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(54) LHRH analogs.

(55) The present invention deals with LHRH analogues which contain cytotoxic moieties and have influence on the release of gonadotropins from the pituitary gland of mammals, including humans. The compounds of this invention are represented by the formula:

X-R¹-R²-R³-Ser-R⁵-R⁶(Q)-Leu-Arg-Pro-R¹⁰-NH₂

wherein

R¹ is pGlu, Pro, D-Nal(2), or D-Phe(4Cl),

R² is His or D-Phe(4Cl),

R³ is Trp, D-Trp or D-Pal(3),

R⁵ is Tyr or Arg,

R⁶ is D-Phe or R⁶, where R⁶ is D-Orn, D-Lys or D-Phe(NH₂),

R¹⁰ is Gly or D-Ala,

X is hydrogen, a lower alkanoyl group of 2-5 carbon atoms or carbamyl,

Q is bis-(2-chloroethyl)amino group provided that R⁶ is D-Phe,

where R⁶ is R⁶,

Q is a complexed metal-containing acyl group having the formula:

[(Q')(A)] or [(Q')(B)₂(A)]

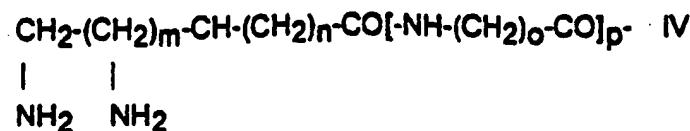
II III

wherein

Q' is Pt(Y)₂, where Y is an anion derived from a pharmaceutically acceptable acid,

A is a diaminoacyl group having the formula

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where

m is 0 or 1,

n and p are 0-10,

o is 1-10,

Q is a non-platinum-group metal, either a main-group metal such as gallium, germanium, and tin, or a transition metal such as titanium, vanadium, iron, copper, cobalt, gold, nickel, cadmium and zinc,

B is a aralkylidene, heteroaralkylidene, cycloalkylidene or heterocycloalkylidene group containing oxygen anion or carboxylate anion at position 2 or 3, and pharmaceutically acceptable salts thereof and methods of use pertaining these compounds.

This invention was made with Government support under Grant Nos. 2RD1 CA40003 and CA40004, awarded by the N.C.I (NIH). The U.S. Government has certain rights in this application. This application is a continuation in part of copending application SN07/280,994 filed October 21, 1988.

- The present invention relates to novel peptides which contain cytotoxic moieties and have influence on the release of gonadotropins from the pituitary in mammals, including humans. More specifically, the present invention relates to analogues of luteinizing hormone-releasing hormone (LHRH), which have the structure:
- $\text{pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH}_2$
- salt thereof, and to pharmaceutical compositions and methods of use pertaining to these analogues.

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DISCUSSION OF THE PRIOR ART

- 16 Hypothalamic luteinizing hormone-releasing hormone (LHRH) controls pituitary synthesis of gonadotropins (LH and FSH) that stimulate the synthesis of sex steroids in the gonads.

Many analogues of this hormone have been prepared and studied in order to find molecule(s) with sufficient biological activity to be clinically useful.

- 18 Significant enhancement of the LH-releasing potency has been obtained by introducing D-amino acids in place of Gly at position 6 and in some analogues also by modification at position 10.

20 The superagonist analogues of LHRH involved in clinical development are represented by general formula A wherein the combination of residues R^6 and R^{10} are as follows:

A pGlu-His-Trp-Ser-Tyr- R^6 -Leu-Arg-Pro- R^{10}

A1 (Leuprolid), R^6 is D-Leu, R^{10} is NH-Et;

- 22 J. A. Vilchez-Martinez et al., Biochem. Biophys. Res. Commun., 59, 1226-1232 (1974).

A2 (Decapeptidyl), R^6 is D-Trp, R^{10} is Gly-NH₂;

D. H. Coy et al., J. Med. Chem., 19, 423-425 (1976).

A3 (Buserelin), R^6 is D-Ser(tBu), R^{10} is NH-Et;

W. Koenig et al., In: R. Walter and J. Meienhofer (eds.),

- 24 Peptides: Chemistry, Structure and Biology. Proceedings of the Fourth American Peptide Symposium. Ann Arbor Science, Ann Arbor, MI, 1975, pp. 883-888.

A4 (Zoladex, ICI-118630), R^6 is D-Ser(tBu), R^{10} is NH-NH-CO-NH₂;

A.S. Dutta et al., J. Med. Chem. 21, 1018-1024 (1978).

A5 (Nafarelin), R^6 is D-Nal(2), R^{10} is Gly-NH₂;

- 26 J.J. Nestor et al., J. Med. Chem., 25, 795-801 (1982).

In addition to agonists, analogues have been prepared which are competitive antagonists to LHRH. In these analogues, agonist activity is reduced by aromatic D-amino acid substitutions at position 2 and 3, and receptor affinity is retained by replacements at positions 1 and 6, in some analogues, also 10, and by acylation of the peptide N-terminus. It has also been shown that replacement of Tyr⁵ by basic residues,

- 28 such as Arg, in combination with a hydrophobic residue at position 6 is helpful. Most of the highly potent antagonists of LHRH are represented by general formula B wherein the combination of residues R^3 , R^5 , R^6 , and R^8 are as follows:

Ac-D-Nal(2)-D-Phe(4Cl)- R^3 -Ser- R^5 - R^6 -Leu- R^8 -Pro-D-Ala-NH₂ B

B1, R^3 is D-Trp, R^5 is Tyr, R^6 is D-Arg, and R^8 is Arg;

- 30 A. Horvath et al., Peptides, 3, 969-971 (1982).

B2, R^3 is D-Pal(3), R^5 is Arg, R^6 is D-Glu(AA), i.e.

4-(p-methoxybenzoyl)-D-2-aminobutyric acid, and R^8 is Arg; J.E. Rivier et al., J. Med. Chem., 29, 1846-1851 (1986).

B3, R^3 is D-Pal(3), R^5 is Lys(nicotinyl), R^6 is D-Lys(nicotinyl) and R^8 is Lys(isopropyl);

- 32 A. Ljungqvist et al., Biochem. Biophys. Res. Commun., 148, 849-856 (1987).

B4, R^3 is D-Trp or D-Pal(3), R^5 is Tyr, R^6 is D-Cit or D-Hcl, and R^8 is Arg;

S. Bajusz et al., Proc. Natl. Acad. Sci. USA, 85, 1637-1641 (1988); U.S. Patent Application SN. 197,153, filed 05/23/88.

- 34 It has been revealed that chronic administration of superagonist analogues of LHRH is at first associated with the expected increase in gonadotropin and steroid secretion, then it leads to the inhibition of pituitary and gonadal functions, to wit, to chemical castration [J. Sandow et al., Fertil. Steril., 20, 205-209(1978); A. J. W. Hsueh and G. F. Erickson, Science, 204, 854 (1979)]. LHRH antagonists exert the same effects but of course, without causing initial stimulation.

In accordance with the above, LHRH superagonist A4 has been found to be as effective as ovariectomy in treatment of rat mammary carcinomas, which is known to contain estrogen receptors [R.I.Nicholson and P.V.Maynard, Br. J. Cancer, 39, 268-273(1979)]. Furthermore, LHRH superagonists, such as A2, have been reported to be of value in treating patients with certain hormone-dependent tumors, such as breast and prostatic cancers [A. V. Schally et al., Cancer Treat. Rep., 68, 281 (1984); H. Parmar et al., Lancet, 2, 1201 (1985)].

Remission of breast or prostate tumor growth after LHRH agonist (A4) administration is apparently mediated by suppression of gonadotropin secretion and the resultant decrease of gonadal steroid hormone secretion [R.I. Nicholson et al., J. Steroid Biochem., 20, 129 (1984)].

Since remission of tumor growth has also been observed in postmenopausal women (in whom ovarian steroid production is minimal) it is supposed that these peptides act directly on breast tumor cells [H. A. Harvey et al., Proc. Am. Assoc. Cancer Res. Am. Soc. Clin. Oncol. 22, 436 (1981)].

In vitro studies have also suggested that LHRH agonists and antagonists may act directly on cancer cells. Namely, it has been demonstrated that certain human breast carcinoma cells contain binding sites for LHRH [K. A. Eidne et al., Science 229, 989-991 (1985)] and that the growth of such cells can be inhibited by LHRH agonist A3 [W. R. Miller et al., Nature 313, 231-233 (1985)]. Another study has revealed that certain LHRH antagonists (e.g. the D-alpha-Me-4-Cl-Phe analogue of B1) bind to several breast cancer cell lines and inhibit incorporation of [³H]thymidine into DNA as well as tumor growth [K. A. Eidne et al., J. Clin. Endocr. Metab. 64, 425-432 (1987)]. Our recent, still unpublished findings have revealed that both agonistic and antagonistic analogues of LHRH bind to human breast cancer cell membranes, and also to the cell membranes of pancreatic tumor which are known to be hormone-independent.

In spite of the above, clinical studies have shown that the duration of remission of tumor growth may be limited as hormonal manipulations do not prevent the ultimate growth of hormone-independent cells [J. T. Isaacs and D. S. Coffey, Cancer Res. 41, 5070-5075 (1981)]. Combination of hormonal therapy with chemotherapy could forestall this phenomenon and prolong survival [J. T. Isaacs, Prostate 5, 1-17 (1984)].

It is especially desirable, in view of this, to provide LHRH analogues which contain cytotoxic moieties. Such compounds can exert the direct or indirect antitumor effects of LHRH agonists or antagonists and, at the same time, act as chemotherapeutic agents targeted to the tumor cells by the peptide portions for which binding sites are present on the cell membranes.

Three LHRH analogues having a cytotoxic moiety, an agonist and two antagonists, have been synthesized so far. In these molecules, Chlorambucil, 4-[4-(bis[2-chloroethyl] amino)phenyl]butyric acid, is linked to complete decapeptide sequences and a nonapeptide fragment, respectively. The agonist analogue, [D-Lys(Chlorambucil)⁶] LHRH [K. Channabasavaiah and J. M. Stewart, Biochem. Biophys. Res. Commun., 88, 1268-1273 (1979)] and one of the antagonists, [Chlorambucil-D-Leu²,D-Leu³,D-Ala⁶]LHRH (2-10) [C. Y. Bowers et al., Biochem. Biophys. Res. Commun., 61, 698-703 (1974)] showed moderate activities, and the other antagonist, [Chlorambucil-D-Phe¹,D-Phe²,D-Trp³⁻⁶]LHRH [K. Channabasavaiah et al., In: E. Gross and J. Meienhofer (eds.), Peptides, Proceedings of the Sixth American Peptide Symposium, Pierce Chem. Co. Rockford, IL, 1979, pp 803-807] was practically inactive.

The peptides of Formula I (X-R¹-R²-R³-Ser-R⁵-R⁶(Q)-Leu-Arg-Pro-R¹⁰-NH₂, see below) of the present invention wherein residue R⁶ carries [(Q')(B)₂(A)] as cytotoxic moiety, can be related to certain cytotoxic organometallic compounds which have recently been reviewed by P. Kopf-Maier [Naturwissenschaften 74, 374-382 (1987)]. For example, a moiety [(Q')(B)₂(A)] wherein Q' is Cu⁺⁺, B is derived from salicylaldehyde, and A is a 2,3 or 2,4-diamino acid residue, is related to the cytotoxic trans-bis(salicylaldoximato) copper(II) [H. O. Elo and P. O. Lumme, Cancer Treat. Rep. 69, 1021-1022 (1985)]. Moieties [(Q')(B)₂(A)] can also comprise B residues which are derived from hydroxy-oxo-compounds possessing cytotoxic activity in their own right. Such hydroxy-oxo-compounds incorporated into [(Q')(B)₂(A)] by means of Q' and A are, for example, pyridoxal 5-phosphate which is a selective inhibitor of oncoviral DNA polymerase [M. J. Modak, Biochem. Biophys. Res. Commun. 71, 180-187 (1976)]. Similarly, in peptides of Formula I of the present invention, moieties [(PtCl₂)(A)] linked to the peptide portion of the compounds of the present invention, are related to the known cytotoxic platinum compounds such as cis-diaminedichloro platinum(II) ("Cisplatin") [B. Rosenberg et al., Nature 222, 385 (1969)].

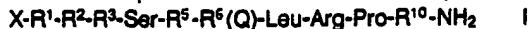
SUMMARY OF THE INVENTION

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The present invention deals with LHRH analogues which possess high agonistic or antagonistic effect and comprise cytotoxic moieties, such as nitrogen mustard, platinum complexes or complexes of metals

derived from the non-platinum-group metal antitumor agents which are represented by inorganic or organometallic compounds containing either main-group metals such as gallium, germanium, and tin, or transition metals such as titanium, vanadium, iron, copper, cobalt and gold, and also nickel, cadmium and zinc, or a cytotoxic compound incorporated as a complex of the above-mentioned metals.

5 The compounds of this invention are represented by Formula I



wherein

R¹ is pGlu, Pro, D-Nal(2), or D-Phe(4Cl),

R² is His or D-Phe(4Cl),

10 R³ is Trp, D-Trp or D-Pal(3),

R⁵ is Tyr or Arg,

R⁶ is D-Phe or R⁶,

where R⁶ is D-Orn, D-Lys or D-Phe(NH₂),

R¹⁰ is Gly or D-Ala,

15 X is hydrogen, a lower alkanoyl group of 2-5 carbon atoms or carbamyl,

Q is bis-(2-chloroethyl)amino group provided that R⁶ is D-Phe,

where R⁶ is R⁶, Q is an acyl group having the formula

20 $[(\text{Q}')(\text{A})] \text{ or } [(\text{Q}'')(\text{B})_2(\text{A})]$



wherein

25 Q' is Pt(Y)₂, where Y is an anion of a pharmaceutically acceptable acid,

A is a diaminoacyl group having the formula



35 where

m is 0 or 1,

n is 0-10,

o and p are 1-10,

Q'' is a non-platinum-group metal, either a main-group metal such as gallium, germanium, and tin, or a transition metal such as titanium, vanadium, iron, copper, cobalt, gold, nickel, cadmium and zinc,

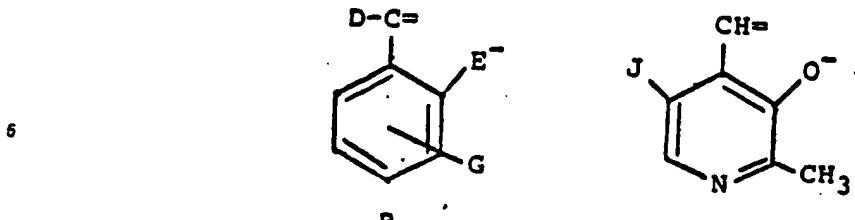
40 B is aralkyldene, heteroaralkyldene, cycloalkyldene or heterocycloalkyldene group containing oxygen anion or carboxylate anion.

Suitably, there are used phenyl loweralkylenes substituted by halo or nitro, preferably chloro or fluoro; lower alkylpyridyl lower alkylidenes, suitably substituted by hydroxy methyl or phosphoxymethyl; furanyl lower alkylidenes, optionally substituted by one or two lower alkyl moieties and, if desired, cycloalkyl lower

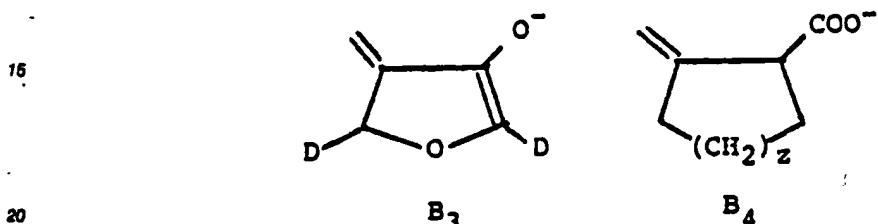
45 alkylidines of 5 to 7 carbon atoms in the ring, optionally substituted by one or two lower alkyl moieties; all of the above cyclic moieties being substituted by hydroxy or carboxy.

Especially preferred are those cyclic compounds in which the substituting oxygen containing function is at position 2 or 3, where it exerts cytotoxic activity either by itself or in combination with the metal, and

50 which is derived from a 2- or 3-hydroxy or carboxy-1-oxo-compound such as substituted or unsubstituted salicylaldehyde, pyridoxal, 4-hydroxy-3-furanone or a 1- or 2-carboxy-cycloalkane of 5-7 carbon atoms that forms a Schiff base with the amino group of A and can combine with a metal ion through its negatively charged oxygen or carboxylate. The preferred cyclic compound may be represented by any of the Formulas B₁-B₄:



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wherein D is methyl or hydrogen,

E is O- or COO-,

25 G is fluoro, chloro or nitro,

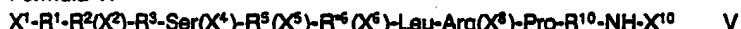
J is hydroxymethyl or phosphoxymethyl,

z is 1, 2 or 3.

The compounds of Formula I can be prepared by a combination of the solid phase technique and the classical (solution) synthesis.

30 Preferably, the compounds of Formula I wherein R⁶ is D-Phe [carrying the bis-(2-chloroethyl)amino group] are prepared by condensation of pentapeptide fragments (1-5) and (6-10).

Compounds of Formula I wherein R⁶ is R⁶ are preferably prepared from intermediate peptides of Formula V:



35 wherein

R¹, R², R³, R⁵, R⁶ and R¹⁰ are as defined hereinabove,

X¹ is a lower alkanoyl group of 2-5 carbon atoms or carbamyl, or provided that R¹ is pGlu, X¹ is hydrogen,

X² is nil or a protecting group for the His imidazole nitrogen,

X⁴ is hydrogen or a protecting group for the Ser hydroxyl group,

40 X⁵ is hydrogen or a protecting group for the Tyr phenolic hydroxyl group, or a protecting group for the guanidino group of Arg,

X⁶ is hydrogen or a protecting group for the Lys, Orn or Phe(NH₂) side chain amino group,

X⁸ is hydrogen or a protecting group for the Arg guanidino group,

X¹⁰ is hydrogen or benzhydryl group incorporated into a resin.

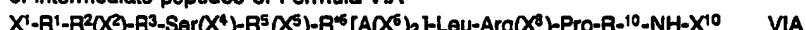
45 The intermediate peptides of Formula V are preferably synthesized by the solid phase method. The ensuing peptides of Formula V wherein X², X⁴, X⁵, X⁶, X⁸, and X¹⁰ are hydrogen, are then acylated with suitably protected A, to yield, after deprotection, intermediate peptides of Formula VI:



wherein

50 X¹, R¹, R², R³, R⁵, R⁶, R¹⁰ and A are as defined hereinabove.

According to another suitable method, intermediate peptides of Formula VI are obtained by deprotection of intermediate peptides of Formula VIA



wherein

55 R¹, R², R³, R⁵, R⁶, R¹⁰, A, X¹, X², X⁴, X⁵, X⁶, X⁸, and X¹⁰ are as defined hereinabove,

which in turn are prepared by the same solid phase method as the intermediate peptides of Formula V with the exception that suitably protected R⁶[A(X⁶)₂] is incorporated in place of suitably protected R⁶(X⁶) in position 6.

The process of preparing the Formula I compounds comprises reacting a peptide of Formula VI with K_2PtCl_4 to obtain a peptide of Formula I wherein residu R^6 carries $[(PtCl_2)(A)]$ as cytotoxic moiety which, if desired, can be converted into $[(PtY_2)(A)]$ by known methods.

5 Alternatively, the process comprises reacting a peptide of Formula VI with a hydroxy- (or carboxyl-) oxo compound and a non-platinum-group metal, as an acetate salt, or with the complex compound they form to yield a peptide of Formula I.

A pharmaceutical composition is provided by admixing the compound of Formula I with pharmaceutically acceptable carrier including microcapsules (microspheres) for delayed delivery.

10 There is also provided a method for relieving complications resulting from the physiological availability of amounts of pituitary gonadotropins in a mammal, in excess of the desired amount, which involves administering to the mammal a gonadotropin antagonizing dose of the compound of Formula I.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

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For convenience in describing this invention, the conventional abbreviations for the amino acids, peptides and their derivatives are used as generally accepted in the peptide art and as recommended by the IUPAC-IUB Commission on Biochemical Nomenclature [European. J. Biochem., 138,9-37(1984)].

20 The abbreviations for the individual amino acid residues are based on the trivial name of the amino acid, e.g. pGlu is pyroglutamic acid, His is histidine, Trp is tryptophane, Ser is serine, Tyr is tyrosine, Lys is lysine, Orn is ornithine, Leu is leucine, Arg is arginine, Pro is proline, Gly is glycine and Ala is alanine, Phe is phenylalanine. Where the amino acid residue has isomeric forms, it is the L-form of the amino acid that is represented unless otherwise expressly indicated. Abbreviations of the uncommon amino acids employed in 25 the present inventions are as follows: D-Met is 4-[bis(2-chloroethyl)amino]-D-phenylalanine, A₂bu is 2,4-diaminobutyric acid, A₂pr is 2,3-diaminopropionic acid, Nal(2) is 3-(2-naphthyl)alanine, D-Pal(3) is 3-(3-pyridyl)alanine, Phe(NH₂) is 4-aminophenylalanine, Phe(4Cl) is 4-chlorophenylalanine.

Peptide sequences are written according to the convention whereby the N-terminal amino acid is on the left and the C-terminal amino acid is on the right.

30 Other abbreviations used are:

AcOH acetic acid

Ac₂O acetic anhydride

Boc tert.butoxycarbonyl

Bzl benzyl

35 CISAL 5-chloro-2-O-1-benzylidene

DCB 2,6-dichlorobenzyl

DCC N,N'-dicyclohexylcarbodiimide

DIC N,N'-diisopropylcarbodiimide

DIEA N,N-diisopropylethylamine

40 DMF dimethylformamide

Eac 6-amino-caproyl

FUR 5,6-dihydro-2,5-dimethyl-4-O-3-furylidene

HOBt 1-hydroxybenzotriazole

HODNP 2,4-dinitrophenol

45 HOPCP pentachlorophenol

HPLC high performance liquid chromatography

IpOH isopropyl alcohol

MeCN acetonitrile

MeOH methyl alcohol

50 PEN 2-COO-2-cyclopentylidene

POL 2-methyl-3-O-5-hydroxymethyl-4-picolyldene

POLP 2-methyl-3-O-5-phosphoxymethyl-4-picolyldene

SAL 2-O-1-benzylidene

TEA triethylamine

55 TFA trifluoroacetic acid

Tos 4-toluenesulfonyl

Z(2-Cl) 2-chloro-benzyloxycarbonyl

Z benzyloxycarbonyl

Especially preferred are LHRH analogues of Formula I
 $X\text{-}R^1\text{-}R^2\text{-}R^3\text{-Ser}\text{-}R^5\text{-}R^6\text{(Q)\text{-}Leu\text{-}Arg\text{-}Pro\text{-}R}^{10}\text{\text{-}NH}_2$

wherein,

R^1 is D-Nal(2), D-Phe(4Cl),

5 R^2 is D-Phe(4Cl),

R^3 is D-Trp or D-Pal(3),

R^5 is Tyr or Arg,

R^6 is D-Phe or R^6 , where D-Lys or D-Orn,

R^{10} is D-Ala,

10 X is acetyl.

Also preferred is the peptide series where R^1 is pGlu,

R^2 is His,

R^3 is Trp,

R^5 is Tyr,

15 R^6 is as defined hereinabove,

R^{10} is Gly, and

X is hydrogen.

With respect to the remaining preferred moieties:

Q is bis-(2-chloroethyl)amino group provided that R^6 is D-Phe;

20 where R^6 is R^6 , Q is an acyl group having the formula

$\{[Q']\}(A)]$ II or $\{[(Q')_2(B)]_2(A)\}$ III

wherein

Q' is PtCl_2 ,

A is L,D-A₂pr,L,D-A₂bu, L,D-A₂pr-Eac or DL-A₂bu-Eac,

25 Q is Cu⁺⁺ or Ni⁺⁺,

B is SAL, CISAL, POL, POLP, FUR or PEN,

The most particularly preferred embodiments are:

1, pGlu-His-Trp-Ser-Tyr-D-Met-Leu-Arg-Pro-Gly-NH₂,

2, Ac-D-Nal(2)-D-Phe(4Cl)-D-Pal(3)-Ser-Arg-D-Met-Leu-Arg-Pro-D-Ala-NH₂,

30 3, pGlu-His-Trp-Ser-Tyr-D-Lys{[(PtCl₂)(DL-A₂pr)]-Leu-Arg-Pro-Gly-NH₂},

4, Ac-D-Phe(4Cl)-D-Phe(4Cl)-D-Trp-Ser-Tyr-D-Lys{[(PtCl₂) (DL-A₂pr)]-Leu-Arg-Pro-D-Ala-NH₂},

5, Ac-D-Nal(2)-D-Phe(4Cl)-D-Trp-Ser-Arg-D-Lys{[(PtCl₂) (DL-A₂pr)]-Leu-Arg-Pro-D-Ala-NH₂},

6, pGlu-His-Trp-Ser-Tyr-D-Lys{[(Cu⁺⁺)(SAL)₂(A₂pr)]-Leu-Arg-Pro-Gly-NH₂},

7, pGlu-His-Trp-Ser-Tyr-D-Lys{[(Cu⁺⁺)(SAL)₂(D-A₂pr)]-Leu-Arg-Pro-Gly-NH₂},

35 8, pGlu-His-Trp-Ser-Tyr-D-Lys{[(Ni⁺⁺)(SAL)₂(A₂pr)]-Leu-Arg-Pro-Gly-NH₂},

9, pGlu-His-Trp-Ser-Tyr-D-Lys{[(Ni⁺⁺)(SAL)₂(D-A₂pr)]-Leu-Arg-Pro-Gly-NH₂},

10, pGlu-His-Trp-Ser-Tyr-D-Lys{[(Cu⁺⁺)(CISAL)₂(A₂pr)]-Leu-Arg-Pro-Gly-NH₂},

11, pGlu-His-Trp-Ser-Tyr-D-Lys{[(Ni⁺⁺)(CISAL)₂(DL-A₂pr)]-Leu-Arg-Pro-Gly-NH₂},

12, Ac-D-Nal(2)-D-Phe(4Cl)-D-Pal(3)-Ser-Arg-D-Lys{[(Cu⁺⁺)(SAL)₂(DL-A₂pr)]-Leu-Arg-Pro-D-Ala-NH₂},

40 13, Ac-D-Nal(2)-D-Phe(4Cl)-D-Trp-Ser-Arg-D-Lys{[(Cu⁺⁺)(SAL)₂(DL-A₂pr)]-Leu-Arg-Pro-D-Ala-NH₂},

14, Ac-D-Nal(2)-D-Phe(4Cl)-D-Pal(3)-Ser-Arg-D-Lys{[(Ni⁺⁺)(SAL)₂(DL-A₂pr)]-Leu-Arg-Pro-D-Ala-NH₂},

15, pGlu-His-Trp-Ser-Tyr-D-Lys{[(Cu⁺⁺)(SAL)₂(DL-A₂bu)]-Leu-Arg-Pro-Gly-NH₂},

16, Ac-D-Nal(2)-D-Phe(4Cl)-D-Trp-Ser-Arg-D-Lys{[(Ni⁺⁺)(SAL)₂(DL-A₂pr)]-Leu-Arg-Pro-D-Ala-NH₂},

17, pGlu-His-Trp-Ser-Tyr-D-Lys{[(Cu⁺⁺)(POLP)₂(LD-A₂bu)]-Leu-Arg-Pro-Gly-NH₂},

45 18, pGlu-His-Trp-Ser-Tyr-D-Lys{[(Cu⁺⁺)(FUR)₂(DL₂A₂Pr)]-Leu-Arg-Pro-Gly-NH₂},

19, pGlu-His-Trp-Ser-Tyr-D-Lys{[(Cu⁺⁺)(POL)₂(DL₂A₂Pr)]-Leu-Arg-Pro-Gly-NH₂},

20, pGlu-His-Trp-Ser-Tyr-D-Lys{[(Cu⁺⁺)(POLP)₂(A₂pr)]-Leu-Arg-Pro-Gly-NH₂},

21, Ac-D-Nal(2)-D-Phe(4Cl)-D-Pal(3)-Ser-Arg-D-Lys{[(Cu⁺⁺)(POLP)₂(DL-A₂pr)]-Leu-Arg-Pro-D-Ala-NH₂},

22, pGlu-His-Trp-Ser-Tyr-D-Lys{[(Cu⁺⁺)(PEN)₂(A₂pr)]-Leu-Arg-Pro-Gly-NH₂},

50 23, Ac-D-Nal(2)-D-Phe(4Cl)-D-Trp-Ser-Arg-D-Lys{[(Cu⁺⁺)(POLP)₂(DL-A₂pr)]-Leu-Arg-Pro-D-Ala-NH₂},

24, Ac-D-Nal(2)-D-Phe(4Cl)-D-Trp-Ser-Arg-D-Lys{[(Cu⁺⁺)(POL)₂(DL-A₂pr)]-Leu-Arg-Pro-D-Ala-NH₂},

25, Ac-D-Nal(2)-D-Phe(4Cl)-D-Pal(3)-Ser-Arg-D-Lys{[(Cu⁺⁺)(POL)₂(DL-A₂pr)]-Leu-Arg-Pro-D-Ala-NH₂},

26, pGlu-His-Trp-Ser-Tyr-D-Lys{[(Cu⁺⁺)(POL)₂(DL-A₂bu)]-Leu-Arg-Pro-Gly-NH₂},

27, pGlu-His-Trp-Ser-Tyr-D-Lys{[(Cu⁺⁺)(SAL)₂(DL-A₂bu-Eac)]-Leu-Arg-Pro-Gly-NH₂},

55 28, pGlu-His-Trp-Ser-Tyr-D-Lys{[(PtCl₂)(A₂bu)]-Leu-Arg-Pro-Gly-NH₂},

29, pGlu-His-Trp-Ser-Tyr-D-Lys{[(PtCl₂)(A₂bu-Eac)]-Leu-Arg-Pro-Gly-NH₂},

In all of the above embodiments, the compound may also be prepared as the addition salts of pharmaceutically acceptable organic or inorganic acids. As examples, but not as limitations, the following

acids may be mentioned hydrochloride, hydrobromide, sulphate, phosphate, fumarate, gluconate, tannate, maleate, acetate, citrate, benzoate, succinate, alginic, pamoate, malate, ascorbat , tartrate, and th like.

5

Assay procedures

The compounds of this invention exhibit a surprisingly powerful effect on gonadotropin release by the pituitary, bind to tumor cell membranes and inhibit [³H]thymidine incorporation into DNA.

10

(a) LHRH inhibiting effect

The ability of compounds to influence LH release in vitro is assayed by using a superfused rat pituitary cell system [S. Vigh and A. V. Schally, Peptides, 5 Suppl. 1, 241-247(1984)].

This effect of the peptides is assayed as follows: Each peptide is perfused through the cells for 9 min (3-ml perfusate) at 3 nM. Immediately after that, a mixture containing the same concentration of peptide and 3 nM LHRH is administered for 3 min. This was followed by four consecutive infusions of 3 nM LHRH for 3 min (1-ml perfusate) at 30-min intervals (30, 60, 90, 120 min). LH content of the 1-ml fractions collected is determined by RIA.

(b) In vivo antiovulatory activity

This activity of the peptides is determined in 4-day-cycling rats as described [A. Corbin and C. W. Beattie, Endocr. Res. Commun. 2, 1-23(1975)].

(c) Affinity for peptides to human prostate and breast cancer cell membranes.

This is determined by using labelled LHRH and [D-Trp⁶]LHRH. The assay is carried out similarly to that described by T. Kadar et al., Proc. Natl. Acad. Sci. USA, 85, 890-894 (1988).

(d) Ability of peptides of Formula I to inhibit incorporation of [³H]thymidine into DNA of monolayer cultures the rat mammary tumor cell line MT-4

This is assayed as described [V. K. Sondak et al., Cancer Research, 44, 1725-1728 (1984); F. Holzel et al., J. Cancer Res. Clin. Oncol. 109, 217-228 (1985); M. Albert et al., J. Cancer Res. Clin. Oncol. 109, 210-216 (1985)].

Synthesis of peptides

45

The peptides of the present invention may be synthesized by any techniques that are known to those skilled in the peptide art. A summary of the many techniques so available may be found in M. Bodanszky, Principles of Peptide Synthesis, Springer-Verlag, 1984. The techniques of exclusively solid-phase synthesis are set forth in the textbook J. M. Stewart and J. D. Young, Solid Phase Peptide Synthesis, Pierce Chem Co., Rockford, IL, 1984 (2nd ed.). Classical solution synthesis is described in detail in the treatise "Methoden der Organischen Chemie" (Houben-Weyl), Vol. 15, Synthese von peptiden, Parts I and II, Georg Thieme Verlag, Stuttgart, 1974.

In general, these methods comprise the sequential addition of one or more suitably protected amino acids to a growing peptide chain. Normally, either the amino or the carboxyl group of the first amino acid is protected by a suitable protecting group.

The protected amino acid can then be either attached to an inert solid support or utilized in solution by adding the next amino acid in the sequence having the complimentary (amino or carboxyl) group suitably protected, under conditions suitable for forming the amide linkage. The protecting group is then removed

from this newly added amino acid residue and the next amino acid (suitably protected) is then added, and so forth. After all the desired amino acids have been linked in the proper sequence, any remaining protecting (and any solid support) are removed sequentially or concurrently, to afford the final peptide. By simple modification of this general procedure, it is possible to add more than one amino acid at a time to a growing peptide chain, for example, by coupling (under conditions which do not racemize chiral centers) a protected pentapeptide with a properly protected another pentapeptide to form, after deprotection, a decapeptide.

10

Preferred Embodiment of Synthesis

A particularly preferred method of preparing compounds of the present invention involves a combination of the solid phase and classical (solution) syntheses.

In this particularly preferred method, the alpha-amino function of the amino acids is protected by an acid sensitive group. Suitable protecting groups are t-butyloxycarbonyl (Boc), benzyloxycarbonyl (Z), t-amyloxycarbonyl and the like, especially Boc.

Particularly preferred side chain protecting groups are, for arginine: p-toluenesulfonyl and nitro, tyrosine: benzyl and 2,6-dichlorobenzyl, serine: benzyl, lysine and ornithine: benzyloxycarbonyl or 2-chlorobenzylloxycarbonyl, histidine: p-toluenesulfonyl or 2,4-dinitrophenyl.

The C-terminal amino acid is attached to a suitable solid support. For the synthesis of peptides with free carboxyl group, suitable solid supports are chloromethyl-polystyrene-divinylbenzene polymer, hydroxymethyl-polystyrene-divinylbenzene polymer and the like, especially chloromethyl-polystyrene-1% divinylbenzene polymer. For the synthesis of peptide amides, a useful support is the benzhydrylaminopolystyrene-2% divinyl benzene polymer. Both chloromethyl resins and benzhydrylamine resins are commercially available.

The attachment to the chloromethyl resin is made by means of reaction of the alpha-amino-protected amino acid, especially Boc-amino acids, as its cesium, triethylamine or similar salt in DMF, MeCN or ethanol at elevated temperature. According to another, especially useful approach, Boc-amino acid is attached to the chloromethyl resin in the presence of KF in DMF at about 60°C for 24 hours with stirring [K. Horiki et al., Chemistry Letters, 1978, 165-168]. The Boc-amino acids is attached to the benzhydrylamine resin by means of a carbodimide, such as DCC or DIC, or carbodimide/HOBt mediated coupling.

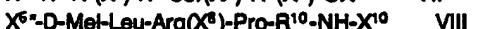
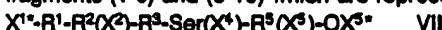
The coupling of successive protected amino acids can be carried out in either an automatic or a manual peptide synthesizer as is well known in the art.

The removal of the Boc group may be performed by an acid in a suitable solvent, preferably by 50% trifluoroacetic acid in CH_2Cl_2 .

Each protected amino acid is preferably introduced in a 2-4 molar, preferably in 3 molar excess, and the coupling is carried out in CH_2Cl_2 or DMF/ CH_2Cl_2 mixtures depending on the solubility of Boc-amino acids at ambient temperature. The coupling agent is normally DIC or other carbodimide either alone or in the presence of HOBt.

At the end of the solid phase synthesis the fully protected peptide is preferably removed from the resin support by treatment with liquid hydrogen fluoride (HF), which not only cleaves the peptide from the resin but also cleaves all remaining the side chain protecting groups.

The peptides of Formula I wherein R⁶ is a D-Phe residue carrying the bis-(2-chloroethyl)amino group, i.e. R⁶(Q) is a 4-[bis(2-chloroethyl)amino]-D-phenylalanine (D-Mel) residue, are preferably synthesized from fragments (1-5) and (6-10) which are represented by Formulas VII and VIII, respectively:



wherein

R¹, R², R³, R⁵, and R¹⁰ are as defined hereinabove,

X¹ is hydrogen, an acyl group of 2-5 carbon atom or carbamyl,

X² is nil or a suitable protecting group for the His imidazole nitrogen, such as Tos or 2,4-dinitrophenyl. The preferred protecting group is Tos.

X⁴ is hydrogen or a suitable protecting group for the hydroxyl group of Ser such as Bzl or DCB. The preferred protecting group is Bzl.

X⁶ is hydrogen or a suitable protecting group for the phenolic hydroxyl group of Tyr; DCB is the preferred group; or X⁸.

X⁵ is hydrogen or benzyl group being incorporated into a solid support.

X^6 is hydrogen or Boc.

X^8 is hydrogen or a suitable protecting group for the guanidino group of Arg such as Tos or nitro. The preferred protecting group is Tos.

X^{10} is hydrogen or benzhydryl group being incorporated into a solid support.

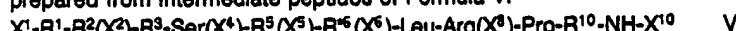
5 Pentapeptide fragment VII and the (8-10) tetrapeptide portion of fragment VIII are preferably prepared by the solid phase method.

Synthesis of fragment VII is commenced with the attachment of protected Arg or Tyr to a chloromethyl resin by using KF in DMF.

The tetrapeptide amide portion of VIII is built up on a benzhydrylamine resin. The free tetrapeptide 10 amide is then coupled with Boc-D-Met to yield, after deprotection, the free pentapeptide amides.

The ensuing free pentapeptides of Formulas VII and VIII wherein X^2 , X^4 , X^5 , X^{5*} , X^6 , X^8 , and X^{10} are hydrogen, are condensed by the DCC/HOBt method to afford, after purification by HPLC, the desired peptides of Formula I wherein D-Met is found at the sixth position.

The peptides of Formula I wherein R^6 is R^6 , that is D-Lys, D-Orn or D-Phe(NH₂) are preferably 15 prepared from intermediate peptides of Formula V:



wherein

R^1 , R^2 , R^3 , R^5 , R^6 , R^{10} , X^1 , X^2 , X^4 , X^5 , X^8 , and X^{10} are as defined hereinabove,

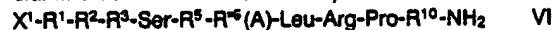
X^6 is a suitable protecting group for side chain amino group of Lys, Orn or D-Phe(NH₂) such as Z, Z(2-Cl);

20 Z(2-Cl) is the preferred protecting group.

The solid phase synthesis of the peptides of Formula V is commenced by the attachment of Boc-protected Gly or D-Ala to a benzhydrylamine resin in CH₂Cl₂. The coupling is carried out using DIC. After the removal of the Boc group, the coupling of successive protected amino acids (each is applied in a 3 molar excess) is carried out in CH₂Cl₂ or in mixtures of DMF/CH₂Cl₂ depending on the solubility of Boc-amino acids. The success of coupling reaction at each stage of the synthesis is preferably monitored by the ninhydrin test as described by Kaiser et al. [Anal. Biochem. 34, 585(1970)].

After the desired amino acid sequence of intermediate peptides of Formula V has been completed, if desired, the N-terminal acetylation is carried out using Ac₂O/ imidazole, and the peptide-resin is then treated with liquid HF in the presence of anisole to yield the peptides of Formula V wherein X^2 , X^4 , X^5 , X^6 , 30 X^8 , and X^{10} are hydrogen.

The ensuing unprotected intermediate peptides of Formula V are then acylated with Boc-protected A diamino acids to obtain, after deprotection, intermediate peptides of Formula VI:



wherein

35 X^1 , R^1 , R^2 , R^3 , R^5 , R^6 , and R^{10} are as defined hereinabove, and A is L,D-2,3-diaminopropyl, L,D-2,4-diaminobutyryl, L,D-2,3-diaminopropyl-6-aminocaproyl or L,D-2,4-diaminobutyryl-6-aminocaproyl.

In an alternate synthesis of intermediate peptides of Formula VI are obtained by deprotection of 40 intermediate peptides of Formula VIA which are prepared by the solid phase method as intermediate peptides having the Formula V, but a suitably protected $R^6(A)$ residue, preferably Boc- $R^6[A(Z)_2]$, incorporated in position 6.

Intermediate peptides of Formula VI are converted into peptides of Formula I wherein residue R^6 is R^6 and it carries [(PtCl₂)(A)] as the cytotoxic moiety by reacting the peptide with an equivalent amount of 45 K₂PtCl₄ in 60-80% aqueous DMF at ambient temperature followed by purification by HPLC. The PtCl₂-containing compounds, if desired, can be converted into their PtY₂-containing congeners by known procedures.

Peptides of Formula I wherein residue R^6 is R^6 and carry [(Q')(B)₂(A)] as cytotoxic moiety containing a non-platinum-group metal ion are obtained from intermediate peptides of Formula VI by reacting with a 50 suitable hydroxy (or carboxy) oxo-compound then a metal salt of a pharmaceutically acceptable acid, preferably the acetate of Cu⁺⁺, Ni⁺⁺, Co⁺⁺, Zn⁺⁺ or, for example, Cd⁺⁺ or with a complex formed by the above-mentioned hydroxy (or carboxy) oxo-compound and the metal itself, in 60-80% aqueous DMF solution the desired peptide-metal-complex is then isolated, preferably by HPLC.

All of the peptides are considered to release gonadotrophin from the pituitary in mammals at very low 55 dosages.

The peptides of the invention are often administered in the form of pharmaceutically acceptable, nontoxic salts, such as acid addition salts. Illustrative of such acid addition salts are hydrochloride, hydrobromide, sulphate, phosphate, fumarate, gluconate, tannate, maleate, acetate, citrate, benzonate,

succinate, alginate, pamoate, malate, ascorbate, tartrate, and the like. If the active ingredient is to be administered in tablet form, the tablet may contain a pharmaceutically acceptable diluent which includes a binder, such as tragacanth, corn starch or gelatin; a disintegrating agent, such as alginic acid and a lubricant, such as magnesium stearate.

5 If administration in liquid form is desired, sweetening and/or flavoring may be used as part of the pharmaceutically-acceptable diluent, and intravenous administration in isotonic saline, phosphate buffer solutions or the like may be effected.

10 The pharmaceutical compositions will usually contain the peptide in conjunction with a conventional, pharmaceutically-acceptable carrier. Usually, the dosage will be from about 1 to about 100 micrograms of the peptide per kilogram of the body weight of the host when given intravenously; oral dosages will be higher. Overall, treatment of subjects with these peptides is generally carried out in the same manner as the clinical treatment using other antagonists of LHRH.

15 These peptides can be administered to mammals intravenously, subcutaneously, intramuscularly, orally, intranasally or intravaginally to achieve fertility inhibition and/or control and also in applications calling for reversible suppression of gonadal activity, such as for the management of precocious puberty or during radiation- or chemo-therapy. Effective dosages will vary with the form of administration and the particular species of mammal being treated. An example of one typical dosage form is a physiological saline solution containing the peptide which solution is administered to provide a dose in the range of about 0.1 to 2.5 mg/kg of body weight. Oral administration of the peptide may be given in either solid form or liquid form.

20 Although the invention has been described with regard to its preferred embodiments, it should be understood that changes and modifications obvious to one having the ordinary skill in this art may be made without departing from the scope of the invention, which is set forth in the claims which are appended thereto. Substitutions known in the art which do not significantly detract from its effectiveness may be employed in the invention.

25

PREPARATION I

30

Boc-D-Met-OPCP

35 D-4-[bis-(2-chloroethyl)amino]phenylalanine, D-Met, (5 mmol) was converted to its Boc derivative as described for the L isomer [H. Kun-hwa and G. R. Marshall, J. Med. Chem. 24, 1304-1310 (1981)] with the exception that di-tert-butyl dicarbonate was used as acylating agent instead of Boc-azide. The oily product, Boc-D-Met, was dissolved in DMF (5 ml) and cooled to 0°C. To the stirred solution triethylamine (5 mmol) and pentachlorophenyl trichloro- acetate [M. Fujino and C. Hatanaka, Chem. Pharm. Bull., 16, 929 (1968)] (5 mmol) were added. After 10-min. stirring, the reaction mixture was diluted with ice-water, and the crystalline material separated was filtered off, washed with ice water and ethanol and, after drying, washed with MeCN and dried again. Boc-D-Met-OPCP thus obtained (about 3 mmol) has a m.p. of 138-140°C.

45

PREPARATION II

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Boc-Tyr(DCB)-OH (1.5 mmol) dissolved in DMF (10 ml) was reacted with a chloromethyl-polystyrene-1% divinyl- benzene resin (Bachem, Torrance, CA) (1 g) and KF (3 mmol) with stirring at 60° for 24 hours. The ensuing Boc-Tyr-O-RESIN (taken as 1 mmol) was coupled sequentially with a 3 molar excess of protected amino acids in accordance with the synthesis program, as follows:

55

STEP	REAGENTS AND OPERATIONS	MIXING TIMES(min.)
5	1 Deprotection: 50% TFA in DCM (twice)	5 and 25
	2 DCM wash	2
	3 2-Propanol wash	1
	4 Neutralization: 10% TEA in DCM	2
	5 MeOH wash	1
	6 Neutralization: 10% TEA in DCM	2
10	7 MeOH wash	1
	8 DCM wash (three times)	2
	9 Coupling:Boc-amino acid in DCM or DMF depending on the solubility of the particular protected amino acid, plus DIC	60-90
	10 MeOH (or DMF then MeOH)wash	2
15	11 DCM wash	2
	12 MeOH wash	2
	13 DCM wash (three times)	2

20 Steps 1-13 complete a coupling cycle for one amino acid. Thus, the resin was treated during successive coupling cycles with Boc-Ser(Bzl), Boc-Trp, Boc-His(Tos), and pGlu (3 mmol of each).

The peptide resin obtained (2.1 g) was treated with 2 ml anisole and 20 ml HF at 0°C for 45 min. After elimination of HF under high vacuum, the peptide-resin remainder was washed with dry diethyl ether and ethyl acetate. The peptide was then extracted with 50% aqueous acetic acid, separated from the resin by filtration, and lyophilized.

25 Crude peptide (686 mg) was purified using a Beckman Prep-350 preparative HPLC system [with a Beckman Type 163 variable wavelength UV detector]. Separations were achieved on a 41.5x250 mm DYNAMAX column packed with spherical C18 silica gel (300 Å pore size, 12 μm particle size) (RAININ Inc. Co. Woburn, MA) with solvents A: 0.1% aqueous TFA and B: 0.1% TFA in 70% aqueous MeCN (referred to as system I in the following) using a gradient of 15-30% B in 30 min. The column eluate was monitored at 220 nm and 280 nm.

30 The peptide thus obtained (350 mg) was judged to be substantially (>95%) pure by using a Hewlett-Packard Model HP-1090 liquid chromatograph. The peptides were chromatographed on a 4.6x250 mm W-Porex 5 μm C18 column (Phenomenex, Rancho Palos Verdes, CA) at a flow rate of 1.2 ml/min. with solvent system I in a linear gradient mode (15-45% B in 60 min). Retention time for the pentapeptide was 12.4 min.

35 The preparation of peptide Ac-Pro-D-Phe(4Cl)-D-Trp-Ser-Tyr is accomplished as described in above with the exception that Boc-D-Trp, Boc-D-Phe(4Cl) and Boc-Pro are incorporated in place of Boc-Trp, Boc-His(Tos) and Boc-D-Nal(2), respectively. The peptide thus obtained as an HPLC retention time of 20.3 min when using system I in a linear gradient mode (30-60% B in 30 min).

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PREPARATION III

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Ac-D-Nal(2)-D-Phe(4Cl)-D-Pal(3)-Ser-Arg (IIIA) and Ac-D-Nal(2)-D-Phe(4Cl)-D-Trp-Ser-Arg (IIIB)

50 Boc-Arg(Tos) (1.5 mmol) was linked to a chloromethyl-polystyrene-1% divinylbenzene resin (Bachem, Torrance, CA) (1 g) by means of KF (3 mmol) in DMF (10 ml) while stirring at 60°C for 24 hours. Boc-Arg(Tos)-O-RESIN thus obtained (taken as 1 mmol) was coupled sequentially with a 3 molar excess of protected amino acids in accordance with the synthesis program given in Preparation II. The amino acid derivatives used during successive coupling cycles were: Boc-Ser(Bzl), Boc-D-Pal(3), Boc-D-Phe(4Cl), and Boc-D-Nal(2). After the amino acid sequence of the pentapeptides has been completed, the terminal Boc group was removed and the N-terminal acetylated by using 10-fold excess of Ac₂O/imidazol to yield peptide resin Ac-D-Nal(2)-D-Phe(4Cl)-D-Pal(3)-Ser(Bzl)-Arg(Tos)-O-RESIN.

55 Proceeding in a similar manner, but incorporating Boc-D-Trp in place of Boc-D-Pal(3) in position 3, there is prepared peptide resin Ac-D-Nal(2)-D-Phe(4Cl)-D-Trp-Ser(Bzl)-Arg(Tos)-O-RESIN.

Peptide-resins were treated with HF as described in Preparation II. The crude products were purified by HPLC with gradient elution (30-60% B in 60 min.). Purified pentapeptides IIIA and IIIB have retention times of 11.8 min. and 25.5 min., respectively, when using solvent system I in a linear gradient mode (30-60% B in 30 min).

- 5 The preparation of peptide Ac-Pro-D-Phe(4Cl)-D-Trp-Ser-Arg is accomplished as described above with the exception that Boc-D-Trp and Boc-Pro are incorporated in place of Boc-D-Pal(3) and Boc-D-Nal(2), respectively. The peptide thus obtained has an HPLC retention time of 13.5 min when using system I in a linear gradient mode (30-60% B in 30 min).

10

PREPARATION IV

15

H-D-Mel-Leu-Arg-Pro-Gly-NH₂ (IVA) and H-D-Mel-Leu-Arg-Pro-D-Ala-NH₂ (IVB)

20 The C-terminal tetrapeptide amide portions of IVA and IVB were built step by step on a benzhydrylamine HCl resin containing about 1 meq NH₂/g (Advanced ChemTech, Louisville, KY) in a reaction vessel for manual solid-phase synthesis starting with Boc-Gly and Boc-D-Ala, respectively, in accordance with the procedures set forth below.

The benzhydrylamine HCl resin (1 g, about 1 mmol), after neutralization with 10% TEA in CH₂Cl₂, was coupled sequentially with 3 molar excess of protected amino acids in accordance with the Schedule as follows:

25

STEP	REAGENTS AND OPERATIONS	MIXING TIMES(min)
30 1	Coupling: Boc-amino acid in DCM or DMF depending on the solubility of the particular protected amino acid, plus DIC	60-90
2	MeOH (or DMF then MeOH) wash	2
3	DCM wash	2
4	MeOH wash	2
5	DCM wash (three times)	2
35 6	Deprotection: 50% TFA in DCM (twice)	5 and 25
7	DCM wash	2
8	2-Propanol wash	1
9	Neutralization: 10% TEA in DCM	2
40 10	MeOH wash	1
11	Neutralization: 10% TEA in DCM	2
12	MeOH wash	1
13	DCM wash (three times)	2

45

Thus, the resin was treated with Boc-Gly, Boc-Pro, Boc-Arg(Tos), and Boc-Leu during successive coupling cycles to yield peptide-resin with structure of Boc-Leu-Arg(Tos)-Pro-Gly-NH-RESIN. Similarly, reacting the resin with Boc-D-Ala, Boc-Pro, Boc-Arg(Tos), and Boc-Leu during successive coupling cycles leads to the peptide resin having the structure of Boc-Leu-Arg(Tos)-Pro-D-Ala-NH-RESIN.

50

The peptide-resins thus obtained were treated with anisole and HF as described in Preparation II. The free peptide amides were extracted with DMF, separated from the resin by filtration, evaporated, and the residue was triturated with MeCN to yield free tetrapeptide amides H-Leu-Arg-Pro-Gly-NH₂.2 HF (390 mg) and H-Leu-Arg-Pro-D-Ala-NH₂.2 HF (400 mg), respectively.

55

To a stirred solution of H-Leu-Arg-Pro-Gly-NH₂.2 HF (240 mg) in DMF (1 ml) cooled to 0°C, DIEA (0.18 ml) and Boc-D-Mel-OPCP (Preparation I, 330 mg) were added. Stirring was continued until dissolution of the activated ester, then the reaction mixture was kept at 0°C overnight. After evaporation under vacuum, the residue was first triturated with diethyl ether then dissolved in 50% aqueous MeCN and subjected to purification by HPLC with solvents A: 0.2% AcOH and B: 0.2% AcOH in 70% aqueous MeCN (referred to as system ii in the following) using a gradient of 30-60% B in 60 min. The pure Boc-protected pentapeptide

thus obtained was treated with 10% HCl in ethyl acetate for 30 min. to yield the HCl salt of H-D-Met-Leu-Arg-Pro-Gly-NH₂, IVA (250 mg).

Proceeding in a similar manner but using tetrapeptide amide H-Leu-Arg-Pro-D-Ala-NH₂.2 HF (250 mg) as amino component, there was prepared the HCl salt of H-D-Met-Leu-Arg-Pro-D-Ala-NH₂, IVB (245 mg).

- 5 HPLC retention times for peptides IVA and IVB were 15.8 min. and 19.4 min., respectively, when using solvent system I in a linear gradient mode (30-60% B in 30 min).

PREPARATION V

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pGlu-His-Trp-Ser-Tyr-D-Lys(DL-A₂pr)-Leu-Arg-Pro-Gly-NH₂ (VA)

15

pGlu-His-Trp-Ser-Tyr-D-Lys(DL-A₂bu)-Leu-Arg-Pro-Gly-NH₂ (VB)

- To a solution of [D-Lys⁶]LHRH [N. C. Nicholas et al., J. Med. Chem., 19, 937-941 (1976)] (323 mg of the TFA salt) in DMF (1 ml) cooled to 0°C, Boc₂-DL-A₂pr (68 mg), HODNP (89 mg), TEA (0.14 ml), and 20 0.04 ml DIC were added then allowed to stand at 0°C overnight. Reaction mixture was concentrated under vacuum, the oily residue was dissolved in 0.1% TFA and diethyl ether and the aqueous phase was subjected to HPLC in solvent system in a linear gradient mode (20-50% solvent B in 60 min). The pure Boc-protected peptide was then treated with 10% HCl in ethyl acetate to yield the HCl salt of [D-Lys(DL-A₂pr)⁶]LHRH (VA) (248 mg).
- 25 Proceeding in a similar manner but using Boc₂-DL-A₂bu (70 mg) as acid-component, there was prepared the HCl salt of [D-Lys(DL-A₂bu)⁶]LHRH (VB) (230 mg). HPLC retention times for peptides VA and VB were 12.3 min. and 12.1 min., respectively, when using solvent system I in a linear gradient mode (15-45% B in 30 min).

30

PREPARATION VI

35

pGlu-His-Trp-Ser-Tyr-D-Lys(A₂pr)-Leu-Arg-Pro-Gly-NH₂ (VI A)

pGlu-His-Trp-Ser-Tyr-D-Lys(A₂bu)-Leu-Arg-Pro-Gly-NH₂ (VI B)

40

The syntheses of peptides VIA and VIB are accomplished as described in Procedure V with the exception that Boc₂-A₂pr and Boc₂-A₂bu are used as acylating components in place of Boc₂-DL-A₂pr and Boc₂-DL-A₂bu, respectively.

45

PREPARATION VII

50

Ac-D-Phe(4Cl)-D-Phe(4Cl)-D-Trp-Ser-Tyr-D-Lys(DL-A₂pr)-Leu-Arg-Pro-D-Ala-NH₂ (VII A) and

Ac-D-Phe(4Cl)-D-Phe(4Cl)-D-Trp-Ser-Tyr-D-Lys(DL-A₂bu)-Leu-Arg-Pro-D-Ala-NH₂ (VII B)

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Preparation of VIIA and VII B were accomplished as described in Preparation VA and VB, respectively, with the exception that [Ac-D-Phe(4Cl)¹,D-Phe(4Cl)²,D-Trp³,D-Lys⁶,D-Ala¹⁰]LHRH [H. D. Coy et al., Endocrinology, 110, 1445-1447 (1982)] (330 mg) was used as amino-component in place of [D-Lys⁶]LHRH to

yield the desired intermediate peptides as HCl salts. The intermediate peptides VIIA and VIIB have HPLC retention times of 20.7 min. and 20.6 min., respectively, when using solvent system I in a linear gradient mode (30-60% B in 30 min).

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PREPARATION VIII

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Ac-Nal(2)-D-Phe(4Cl)-D-Pal(3)-Ser-Arg-D-Lys(DL-A₂pr)-Leu-Arg-Pro-D-Ala-NH₂ (VIIIA)

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Ac-Nal(2)-D-Phe(4Cl)-D-Trp-Ser-Arg-D-Lys(DL-A₂pr)-Leu-Arg-Pro-D-Ala-NH₂ (VIIIB) and

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The syntheses of VIIIA, VIIIB and VIIIC were commenced with the preparation of the linear decapeptide precursors containing D-Lys or D-Orn residue at position 6. These precursor peptides were prepared by the solid-phase technique on a benzhydrylamine HCl resin in accordance with the procedures set forth in the Schedule of Preparation IV.

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Thus, the resin (0.5 g containing about 0.5 mmole NH₂) was treated during the ten successive coupling cycles with Boc-D-Ala, Boc-Pro, Boc-Leu, Boc-Arg(Tos), Boc-Lys[Z(2-Cl)], Boc-Arg(Tos), Boc-Ser(Bzl), Boc-D-Pal(3), Boc-D-Phe(4Cl), Boc-D-Nal(2) and finally with Ac₂O/imidazole to yield a peptide-resin which was then treated with HF and anisole to afford the free, D-Lys-containing decapeptide precursor of VIIIA: Ac-D-Nal(2)-D-Phe(4Cl)-D-Pal(3)-Ser-Arg-D-Lys-Leu-Arg-Pro-D-Ala-NH₂ (540 mg of the TFA salt).

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Proceeding in a similar manner but incorporating Boc-D-Trp in place of Boc-D-Pal(3) at position 3, there was prepared the free, D-Lys-containing decapeptide precursor of VIIIB:

Ac-D-Nal(2)-D-Phe(4Cl)-D-Trp-Ser-Arg-D-Lys-Leu-Arg-Pro-D-Ala-NH₂ (500 mg of the TFA salt),

Again proceeding as above but introducing Boc-D-Orn(Z) in place of Boc-D-Lys[Z(2-Cl)] at position 6, there was prepared the free, D-Orn-containing decapeptide precursor of VIIIC:

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Ac-D-Nal(2)-D-Phe(4Cl)-D-Pal(3)-Ser-Arg-D-Orn-Leu-Arg-Pro-D-Ala-NH₂ (520 mg of the TFA salt).

The decapeptide precursors were acylated with Boc₂-DL-A₂pr and the Boc-protected undecapeptides obtained were deprotected as described in Preparation V to give the HCl salt of intermediate peptides VIIIA, VIIIB, and VIIIC. HPLC retention times of peptides VIIIA, VIIIB and VIIIC were 10.7 min, 17.7 min and 11.0 min, respectively, when using solvent system I in a linear gradient mode (30-60% B in 30 min).

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PREPARATION IX

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Boc₂-DL-A₂bu-Eac (IXA), Boc₂-A₂bu-Eac (IXB)

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Boc₂-DL-A₂pr-Eac (IXC) and Boc₂-A₂pr-Eac (IXD)

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To a mixed anhydride prepared from Boc₂-DL-A₂bu (0.64 g) and ethyl chloroformate (0.2 ml) in the presence of TEA (0.28 ml) in DMF solution (4 ml), %-amino-caproic acid methyl ester hydrochloride (0.4 g) and TEA (0.31 ml) in chloroform (4 ml) were added with stirring at -10°C. Stirring was continued for 2 hours at 0°C-5°C then the reaction mixture was concentrated to about 5 ml under reduced pressure. The residue was dissolved in water and ethyl acetate, the organic layer was successively washed with 1 M KHSO₄, water, and a saturated NaHCO₃ solution, then dried over Na₂SO₄ and evaporated. The ensuing dipeptide

methyl ester, $\text{Boc}_2\text{-DL-A}_2\text{bu-Eac-OMe}$, was dissolved in methanol (5 ml) and 1 M sodium hydroxide (3 ml). After one hour, the mixture was acidified with 1 M KHSO_4 (3 ml) and extracted with ethyl acetate. The ethyl acetate solution was washed with water, dried over Na_2SO_4 , and evaporated to yield $\text{Boc}_2\text{-DL-A}_2\text{bu-Eac}$ (IXA) (0.8 g).

- 5 Proceeding in a similar manner but using $\text{Boc}_2\text{-A}_2\text{bu}$ (0.64 g), $\text{Boc}_2\text{-DL-A}_2\text{pr}$ (0.81 g) and $\text{Boc}_2\text{-A}_2\text{pr}$ (0.81 g) as acid component, there are prepared $\text{Boc}_2\text{-A}_2\text{bu-Eac}$ (IXB), $\text{Boc}_2\text{-DL-A}_2\text{pr-Eac}$ (IXC) and $\text{Boc}_2\text{-A}_2\text{pr-Eac}$ (IXD), respectively.

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PREPARATION X

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pGlu-His-Trp-Ser-Tyr-D-Lys(DL-A₂pr-Eac)-Leu-Arg-Pro-Gly-NH₂ (XA)

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The synthesis of XA and XB was accomplished as described in Preparation V with the exception that $\text{Boc}_2\text{-DL-A}_2\text{pr-Eac}$ (Preparation IXC) (90 mg) and $\text{Boc}_2\text{-DL-A}_2\text{bu-Eac}$ (Preparation IXA) (95 mg) were used in place of $\text{Boc}_2\text{-DL-A}_2\text{pr}$ and $\text{Boc}_2\text{-A}_2\text{bu}$, respectively. After purification by HPLC and deblocking with HCl in ethyl acetate, the desired products, [D-Lys(DL-A₂pr-Eac)⁶]LHRH (260 mg) and [D-Lys(DL-A₂bu-Eac)-⁶]LHRH (260 mg), have HPLC retention times of 14.2 min and 14.4 min, respectively.

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PREPARATION XI

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Boc-D-Lys(Z₂-A₂pr) (XIA) and Boc-D-Lys(Z₂-A₂bu) (XIB)

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To a mixed anhydride prepared from $Z_2\text{-A}_2\text{pr}$ (0.72 g) and ethyl chloroformate (0.2 ml) in the presence of TEA (0.28 ml) in DMF solution (4 ml), Boc-D-Lys (0.5 g) and (0.3 ml) TEA in 50% aqueous DMF (4 ml) were added with stirring at 0°C. After 2 hours stirring at 0°C, the reaction mixture was concentrated to an oil under reduced pressure, dissolved in water and ethyl acetate, acidified with 1 M KHSO_4 . The organic phase was washed with water, then dried over Na_2SO_4 and evaporated under vacuum to yield $\text{Boc-D-Lys}(Z_2\text{-A}_2\text{pr})$ (XIA) (1.1g).

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Proceeding in a similar manner but using $Z_2\text{-A}_2\text{bu}$ (0.78 g) as acid component, there was prepared $\text{Boc-D-Lys}(Z_2\text{-A}_2\text{bu})$ (1.1 g) (XIB).

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PREPARATION XII

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pGlu-His-Trp-Ser-Tyr-D-Lys(A₂pr)-Leu-Arg-Pro-Gly-NH₂ (VIA)

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pGlu-His-Trp-Ser-Tyr-D-Lys(A₂bu)-Leu-Arg-Pro-Gly-NH₂ (VIB)

In this Procedure compounds VIA and VIB were built step by step on a benzhydrylamine HCl resin containing about 1 meq NH_2/g (Advanced ChemTech, Louisville, KY) in a reaction vessel for manual solid-phase synthesis starting with Boc-Gly in accordance with the procedures set forth below.

The benzhydrylamine HCl resin (1 g, about 1 mmol), after neutralization with 10% TEA in CH₂Cl₂, was coupled sequentially with 3 molar excess of protected amino acids in accordance with the Schedule given in Procedure IV.

Thus, the resin was treated with Boc-Gly, Boc-Pro, Boc-Arg(Tos), Boc-Leu, Boc-D-Lys(Z₂-A₂pr) (Preparation XIA), Boc-Tyr(DCB), Boc-Ser(Bzl), Boc-His(Z), and pGlu during successive coupling cycles to yield peptide-resin with structure of pGlu-His(Tos)-Trp-Ser(Bzl)-Tyr(DCB)-D-Lys(Z₂-A₂pr)-Leu-Arg(Tos)-Pro-Gly-NH₂-RESIN.

Proceeding in a similar manner, but reacting the resin with Boc-Gly, Boc-Pro, Boc-Arg(Tos), Boc-Leu, Boc-D-Lys(Z₂-A₂bu) (Preparation XIB), Boc-Tyr(DCB), Boc-Ser(Bzl), Boc-His(Tos), and pGlu during successive coupling cycles leads to the peptide resin having the structure of pGlu-His(Tos)-Trp-Ser(Bzl)-Tyr(DCB)-D-Lys(Z₂-A₂bu)-Leu-Arg(Tos)-Pro-Gly-NH-RESIN.

The peptide-resins thus obtained were treated with anisole and HF, and the crude free peptides were isolated as described in Preparation II. Thereafter the crude peptides (1.2-1.4g) were subjected to purification by HPLC as described in Preparation II.

Peptides VIA and VIB thus obtained (0.7-0.8 g) were judged to be substantially (>95%) pure. HPLC retention times for peptides VIA and VIB were 12.4 min and 12.2 min, respectively, when using solvent system I in a linear gradient mode (15-45% B in 30 min).

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PREPARATION XIII

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Ac-D-Nal(2)-D-Phe(4Cl)-D-Pal(3)-Ser-Arg-D-Lys(A₂pr)-Leu-Arg-Pro-D-Ala-NH₂ (XIIIA) and

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Compounds XIIIA and XIIIB were built step by step on a benzhydrylamine HCl resin containing about 1 meg NH₂/g (Advanced ChemTech, Louisville, KY) in a reaction vessel for manual solid-phase synthesis starting with Boc-D-Ala in accordance with the procedures set forth below.

The benzhydrylamine HCl resin (1 g, about 1 mmol), after neutralization with 10% TEA in CH₂Cl₂, was coupled sequentially with 3 molar excess of protected amino acids in accordance with the Schedule given in Procedure IV. Thus, the resin was treated with Boc-D-Ala, Boc-Pro, Boc-Arg(Tos), Boc-Leu, Boc-D-Lys(Z₂-A₂pr) (Preparation XIA), Boc-Arg(Tos), (Boc-Ser(Bzl), Boc-D-Pal(3), Boc-D-Phe(4Cl), and Boc-D-Nal(2). After the amino acid sequence of the decapeptide has been completed, the terminal Boc group was removed and the N-terminal was acetylated by using 10-fold excess of AC₂O and imidazole to yield peptide-resin with structure of Ac-D-Nal(2)-D-Phe(4Cl)-D-Pal(3)-Ser(Bzl)-Arg(Tos)-D-Lys(Z₂-A₂pr)-Leu-Arg(Tos)-Pro-D-Ala-NH-RESIN.

Proceeding in a similar manner, but reacting the resin with Boc-D-Ala, Boc-Pro, Boc-Arg(Tos), Boc-Leu, Boc-D-Lys(Z₂-A₂bu) (Preparation XIB), Boc-Arg(Tos), Boc-Ser(Bzl), Boc-D-Pal(3), Boc-D-Nal(2), and AC₂O/imidazole during successive coupling cycles leads to the peptide resin having the structure of Ac-D-Nal(2)-D-Phe(4Cl)-D-Pal(3)-Ser(Bzl)-Arg(Tos)-D-Lys(Z₂-A₂bu)-Leu-Arg(Tos)-Pro-D-Ala-NH-RESIN.

The peptide-resins thus obtained were treated with anisole and HF, and the crude free peptides were isolated as described in Preparation II. Thereafter the crude peptides (1.3-1.4 g) were subjected to purification by HPLC using solvent system I in a linear gradient mode (20-50 %B in 60 min). Peptides XIIIA and XIIIB thus obtained (0.7-0.8 g) were judged to be substantially (>95%) pure. HPLC retention times for peptides XIIIA and XIIIB were 10.8 min and 10.6 min, respectively, when using solvent system I in a linear gradient mode (30-60% B in 30 min).

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PREPARATION XIV

Ac-D-Nal(2)-D-Phe(4Cl)-D-Trp-Ser-Arg-D-Lys(A₂pr)-Leu-Arg-Pro-D-Ala-NH₂ (XIVA) and

Ac-D-Nal(2)-D-Phe(4Cl)-D-Trp-Ser-Arg-D-Lys(A₂bu)-Leu-Arg-Pro-D-Ala-NH₂ (XIVB)

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The synthesis of XIVB was accomplished as described in Preparation XIII for the synthesis of XIIIIA and XIIIIB with the exception that Boc-D-Trp was incorporated in place of Boc-D-Pal(3) in position 3. HPLC retention times of XIVB were 17.5 min and 17.7 min, respectively when using solvent system I in a linear gradient mode (30-60% B in 30 min).

PREPARATION XV

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Ac-D-Nal(2)-D-Phe(4Cl)-D-Pal(3)-Ser-Arg-D-Lys(A₂pr-Eac)-Leu-Arg-Pro-D-Ala-NH₂ (XVA)

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AcNal(2)-D-Phe(4Cl)-D-Trp-Ser-Arg-D-Lys(A₂bu-Eac)-Leu-Arg-Pro-D-Ala-NH₂ (XVB)

The synthesis of XVA and XVB was accomplished as described in Preparation VIII with the exception that Boc₂-A₂pr-Eac (Preparation IXD) (90 mg) and Boc₂-A₂bu-Eac (Preparation IXB) (95 mg) were used in place of Boc₂-DL-A₂pr and Boc₂-DL-A₂bu, respectively. After purification by HPLC and deblocking with HCl in ethyl acetate, the desired products, XVA (226 mg) and XVB (247 mg), have HPLC retention times of 11.4 min and 11.7 min, respectively using solvent system I in a linear gradient mode (30-60% B in 30 min).

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EXAMPLE I

The synthesis of peptide pGlu-His-Trp-Ser-Tyr-D-Mel-Leu-Arg-Pro-Gly-NH₂ was accomplished by coupling of pentapeptides pGlu-His-Trp-Ser-Tyr-OH (Preparation II, 70 mg) and H-D-Mel-Leu-Arg-Pro-Gly-NH₂ (Preparation IV, 85 mg of the HCl salt) in DMF (0.2 ml containing 0.02 ml DIEA) by means of DCC (30 mg) in the presence of HOBr (20 mg) at 0°C for 24 hours. The reaction mixture was concentrated under vacuum. The crude material thus obtained is purified on a Beckman HPLC system (Typ 142) using a DYNAMAX Macro 21.2x250 mm column (300 Å pore size, 12 m particle size) with solvent system I in a linear gradient mode (30-60% B in 60 min) to isolate the desired [D-Mel⁶]LHRH (65 mg) with a HPLC retention time of 11.4 min when using solvent system I in a linear gradient mode (40-80% B in 40 min).

EXAMPLE II

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The synthesis of peptide Ac-D-Nal(2)-D-Phe(4Cl)-D-Pal(3)-Ser-Arg-D-Mel-Leu-Arg-Pro-D-Ala-NH₂ was accomplished by coupling of pentapeptides Ac-D-Nal(2)-D-Phe(4Cl)-D-Pal(3)-Ser-Arg-OH (Preparation IIIA, 85 mg) and H-D-Mel-Leu-Arg-Pro-D-Ala-NH₂ (Preparation IV, 85 mg) in DMF solution as described in Example I. The reaction mixture was subjected to HPLC using solvent system II in a linear gradient mode (30-60% B in 60 min) to afford the desired [Ac-D-Nal(2)¹,D-Phe(4Cl)²,D-Pal(3)³,Arg⁵,D-Mel⁶,D-Ala¹⁰]LHRH (60 mg) with a HPLC retention time of 19.2 min when using solvent system I in a linear gradient mode (40-80% B 40 min).

The synthesis of peptide Ac-Pro-D-Phe(4Cl)-D-Trp-Ser-Tyr-D-Mel-Leu-Arg-Pro-Gly-NH₂ is accomplished by coupling of pentapeptides Ac-Pro-D-Phe(4Cl)-D-Trp-Ser-Tyr-OH (Preparation II, 80 mg) and H-D-Mel-Leu-Arg-Pro-Gly-NH₂ (Preparation IV, 80 mg) in DMF solution as described in Example I. The reaction mixture is subjected to HPLC using solvent system II in a linear gradient mode (20-50% B in 60 min) to afford the desired [Ac-Pro¹,D-Phe(4Cl)²,D-Trp³,D-Mel⁶]LHRH (65 mg) with a HPLC retention time of 25.2

min when using solvent system I in a linear gradient mode (40-80% B 40 min).

The synthesis of peptide Ac-Pro-D-Phe(4Cl)-D-Trp-Ser-Arg-D-Mel-Leu-Arg-Pro-Gly-NH₂ is accomplished by coupling of pentapeptides Ac-Pro-D-Phe(4Cl)-D-Trp-Ser-Arg-OH (Preparation III, 80 mg) and H-D-Mel-Leu-Arg-Pro-Gly-NH₂ (Preparation IV, 80 mg) in DMF solution as described in Example I. The reaction mixture is subjected to HPLC using solvent system II in a linear gradient mode (20-50% B in 60 min) to afford the desired [Ac-Pro¹,D-Phe^(4Cl)²,D-Trp³,Arg⁵,D-Mel⁶]LHRH (60 mg) with a HPLC retention time of 20.7 min when using solvent system I in a linear gradient mode (40-80% B 40 min).

The synthesis of peptide Ac-D-Nal(2)-D-Phe(4Cl)-D-Trp-Ser-Arg-D-Mel-Leu-Arg-Pro-D-Ala-NH₂ is accomplished by coupling of pentapeptides Ac-D-Nal(2)-D-Phe(4Cl)-D-Trp-Ser-Arg-OH (Preparation III, 85 mg) and H-D-Mel-Leu-Arg-Pro-D-Ala-NH₂ (Preparation IV, 85 mg) in DMF solution as described in Example I. The reaction mixture is subjected to HPLC using solvent system II in a linear gradient mode (40-70% B in 60 min) to afford the desired [Ac-D-Nal⁽²⁾,D-Phe^(4Cl)²,D-Trp³,Arg⁵,D-Mel⁶,D-Ala¹⁰]LHRH (65 mg) with a HPLC retention time of 27.1 min when using solvent system I in a linear gradient mode (40-80% B 40 min).

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EXAMPLE III

The peptide pGlu-His-Trp-Ser-Tyr-D-Lys[(PtCl₂)(DL-A₂pr)]-Leu-Arg-Pro-Gly-NH₂ was prepared by reacting [D-Lys(DL-A₂pr)⁶] LHRH (Preparation V, 60 mg of the HCl salt) in 0.2 ml DMF with sodium acetate (10 mg) and potassium chloroplatinate (18 mg) dissolved in 0.2 ml water for 24 hours. Thereafter the reaction mixture was diluted with water and subjected to HPLC as in Example I using solvent system II in a linear gradient mode (15-55% B in 40 min). The desired Pt-complex [D-Lys[(PtCl₂)(DL-A₂pr)]⁶]LHRH (45 mg) has HPLC retention time of 10.9 min when using solvent system I in a linear gradient mode (20-60% B in 40 min).

The peptides pGlu-His-Trp-Ser-Tyr-D-Lys[(PtCl₂)(A₂bu)]-Leu-Arg-Pro-Gly-NH₂ and pGlu-His-Trp-Ser-Tyr-D-Lys[(PtCl₂)(A₂bu-Eac)]-Leu-Arg-Pro-Gly-NH₂ are prepared as described in Example III with the exception that [D-Lys(A₂bu)⁶]LHRH (Preparation VI) and [D-Lys(A₂bu-Eac)⁶] LHRH (Preparation XB), respectively, are used in place of [D-Lys⁶] LHRH as starting material. The ensuing Pt-complexes [D-Lys-(PtCl₂)(A₂bu)⁶]LHRH and [D-Lys[(PtCl₂)(A₂bu-Eac)]⁶]LHRH have HPLC retention times of 11.6 min and 13.6 min, respectively when using solvent system I in a linear gradient mode (20-60% B in 40 min).

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EXAMPLE IV

The synthesis of peptide Ac-D-Phe(4Cl)-D-Phe(4Cl)-D-Trp-Ser-Tyr-D-Lys[(PtCl₂)(DL-A₂pr)]-Leu-Arg-Pro-D-Ala-NH₂ was accomplished as described in Example III with the exception that [Ac-D-Phe(4Cl)¹,D-Phe(4Cl)²,D-Trp³,D-Lys(DL-A₂pr)⁶,D-Ala¹⁰]LHRH (Preparation VII, 65 mg of the HCl salt) was used as starting material in place of [D-Lys(DL-A₂pr)⁶]LHRH. The desired peptide-Pt-complex [Ac-D-Phe(4Cl)¹,D-Phe(4Cl)²,D-Trp³,D-Lys[(PtCl₂)(DL-A₂pr)]⁶,D-Ala¹⁰]LHRH (50 mg) has HPLC retention time of 35.5 min. under conditions given in Example III.

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EXAMPLE V

The synthesis of peptide Ac-D-Nal(2)-D-Phe(4Cl)-D-Trp-Ser-Arg-D-Lys[(PtCl₂)(DL-A₂pr)]-Leu-Arg-Pro-D-Ala-NH₂ was accomplished as described in Example III with the exception that [Ac-D-Nal(2),D-Phe(4Cl)²,D-Trp³,Arg⁵,D-Lys(DL-A₂pr)⁶,D-Ala¹⁰]LHRH (Preparation VIII B, 66 mg) was used as starting material in place of [D-Lys(DL-A₂pr)⁶] LHRH. The desired peptide Pt-complex obtained (45 mg) has HPLC retention time of 32.6 min. under conditions given in Example III.

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EXAMPLE VI

Peptide pGlu-His-Trp-Ser-Tyr-D-Lys[(Cu⁺⁺)(SAL)-2(A₂pr)]-Leu-Arg-Pro-Gly-NH₂ and the corresponding

D-A₂pr-containing isomer were prepared by reacting [D-Lys(DL-A₂pr)]LHRH (Preparation VA, 36 mg of the HCl salt) with bis(salicyl aldehydato)copper(II) (7 mg) [Y. Nakao and A. Nakahara, Bull. Chem. Soc. Japan, 46, 187 (1973)] in 90% aqueous DMF containing sodium acetate (4 mg) for 48 hours, then concentrated under vacuum to a small volume and subjected to HPLC. Purification is carried out on a Beckman HPLC system (Typ. 142) using a 10x250 mm VYDAC Protein & Peptide C-18 column (300 Å pore size, 5 μm particle size) with a solvent system consisting of A: 0.1 M ammonium acetate (pH 7), B: 0.1 M ammonium acetate in 65% aqueous MeCN (referring to as system III) in a linear gradient mode (15-45% B in 30 min) to yield the desired LHRH-peptide-copper-complex with L-A₂pr residue (8 mg) and D-A₂pr residue (9 mg), respectively. HPLC retention times for [D-Lys(Cu⁺⁺)(SAL)₂(L-A₂pr)]⁶]LHRH and its D-A₂pr-containing isomer were 16.4 min and 17.8 min, respectively (solvent system III using linear gradient elution with 30-75% B in 30 min).

EXAMPLE VII

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Peptide pGlu-His-Trp-Ser-Tyr-D-Lys[(Cu⁺⁺)(SAL)₂(A₂pr)]-Leu-Arg-Pro-Gly-NH₂ was prepared as described in Example VI with the exception that [D-Lys(A₂pr)]⁶]LHRH (Preparation VIA, 36 mg of the HCl salt) was used in place of [D-Lys(DL-A₂pr)]⁶]LHRH. The desired product has HPLC retention time of 16.3 min.

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EXAMPLE VIII

25 Peptide pGlu-His-Trp-Ser-Tyr-D-Lys[(Ni⁺⁺)(SAL)₂(A₂pr)]-Leu-Arg-Pro-Gly-NH₂ and its D-A₂pr-containing isomer were prepared as described in Example VI with the exception that bis(salicyl aldehydato)nickel(II) (Aldrich, 7 mg) was used in place of bis(salicyl aldehydato) copper(II). The crude product was purified by HPLC as described in Example VI to yield [D-Lys(Ni⁺⁺)(SAL)₂(L-A₂pr)]⁶]LHRH (7 mg) and [D-Lys(Ni⁺⁺)(SAL)₂(D-A₂pr)]⁶]LHRH (8 mg), having HPLC retention times of 17.5 min and 18.3 min, respectively.

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EXAMPLE IX

35 The synthesis of peptide pGlu-His-Trp-Ser-Tyr-D-Lys[(Cu²⁺)(CISAL)₂(DL-A₂pr)]-Leu-Arg-Pro-Gly-NH₂ was performed by reacting [D-Lys(DL-A₂pr)]⁶]LHRH (Preparation V, 36 mg) in DMF (0.2 ml) at pH 8 (adjusted with sodium hydroxide and sodium acetate) with 5-chloro-salicylaldehyde (4 mg) then, after standing at room temperature for 1 hour, with copper(II) acetate (3 mg in 0.05 ml water). The crude product was purified by HPLC under conditions given in Example VI to yield [D-Lys(Cu⁺⁺)(CISAL)₂(DL-A₂pr)]⁶]LHRH (16 mg, a mixture of the two diastereomers), having HPLC retention times of 23.5 min and 24.4 min when using solvent system III in a linear gradient mode (30-75% B in 30 min).

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EXAMPLE X

The synthesis of peptide pGlu-His-Trp-Ser-Tyr-D-Lys [(Ni⁺⁺)(CISAL)₂(D-A₂pr)]-Leu-Arg-Pro-Gly-NH₂ was accomplished as described in Example IX with the exception that [D-Lys(DL-A₂pr)]⁶]LHRH was reacted with nickel(II) acetate (3 mg) in place of copper(II) acetate. After purification by HPLC the product, [D-Lys(Ni⁺⁺)(CISAL)₂(DL-A₂pr)]⁶]LHRH (17 mg, a mixture of the two diastereomers) has retention times of 23.1 min. and 23.8 min.

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EXAMPLE XI

Peptide Ac-D-Nal(2)-D-Phe(4Cl)-D-Pal(3)-Ser-Arg-D-Lys[(Cu⁺⁺)(SAL)₂(DL-A₂pr)]-Leu-Arg-Pro-D-Ala-NH₂ was prepared by reacting [Ac-D-Nal(2)¹, D-Phe(4Cl)², D-Pal(3)³, Arg⁴, D-Lys(DL-A₂pr)⁶, D-Ala¹⁰]LHRH

(Preparation VIII, 40 mg of the TFA salt) in 80% aqueous DMF with bis(salicyl aldehydato)copper(II) (7 mg) in the presence of sodium acetate (4 mg) for 24 hours. The reaction mixture was then subjected to purification by HPLC with solvents A: 0.1 M ammonium acetate (pH 7) and B: 0.1 M ammonium acetate in 70% aqueous isopropanol (referred to as system iv below) using gradient elution (25-50% B in 35 min).

5 Pure [Ac-D-Nal(2)¹, D-Phe(4Cl)²,D-Pal(3)³,Arg⁵, D-Lys(Cu⁺⁺)(SAL)₂(DL-A₂Pr)⁶,D-Ala¹⁰]LHRH (38 mg) has a HPLC retention time of 9.5 min. when using solvent system iv in a linear gradient mode (45-90% B in 30 min).

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EXAMPLE XII

Peptide Ac-D-Nal(2)-D-Phe(4Cl)-D-Trp-Ser-Arg-D-Lys[(Cu⁺⁺)(SAL)₂(DL-A₂pr)]-Leu-Arg-Pro-D-Ala-NH₂ was prepared as described in Example XI with the exception that [Ac-D-Nal(2)¹,D-Phe(4Cl)²,D-Trp³,Arg⁵,D-Lys(DL-A₂pr)⁶, D-Ala¹⁰]LHRH (Preparation VIII, 40 mg of a TFA salt) was used as starting material in place of the corresponding D-Pal(3)³ analog to yield the desired compound, [Ac-D-Nal(2)¹,D-Phe(4Cl)²,D-Trp³,Arg⁵,D-Lys(Cu⁺⁺)(SAL)₂(DL-A₂pr)⁶,D-Ala¹⁰]LHRH (40 mg) with HPLC retention time of 11.7 min. under conditions given in Example XI.

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EXAMPLE XIII

Peptide Ac-D-Nal(2)-D-Phe(4Cl)-D-Pal(3)-Ser-Arg-D-Lys[(Ni⁺⁺)(SAL)₂(DL-A₂pr)]-Leu-Arg-Pro-D-Ala-NH₂ was prepared as described in Example XI with the exception that bis(salicylaldehydato)nickel(II) (7 mg) was used as complex forming agent in place of bis(salicylaldehydato) copper(II). The pure product, [Ac-D-Nal(2)¹,D-Phe(4Cl)²,D-Pal(3)³,Arg⁵,D-Lys[(Ni⁺⁺)(SAL)₂(DL-A₂pr)⁶,D-Ala¹⁰]LHRH (34 mg) has HPLC retention time of 10.0 min. under conditions given in Example XI.

30

EXAMPLE XIV

Peptide pGlu-His-Trp-Ser-Tyr-D-Lys[(Cu⁺⁺)(SAL)₂(DL-A₂bu)]-Leu-Arg-Pro-Gly-NH₂ was prepared as described in Example VI with the exception that [D-Lys(DL-A₂bu)⁶]LHRH (Preparation VB, 36 mg of the HCl salt) was used in place of [D-Lys(DL-A₂pr)⁶]LHRH. The desired product has HPLC retention time of 17.7 min.

40

EXAMPLE XV

Peptide Ac-D-Nal(2)-D-Phe(4Cl)-D-Trp-Ser-Arg-D-Lys[(Ni⁺⁺)(SAL)₂(DL-A₂pr)]-Leu-Arg-Pro-D-Ala-NH₂ was prepared by reacting Ac-D-Nal(2)-D-Phe(4Cl)-D-Trp-Ser-Arg-D-Lys(DL-A₂pr)-Leu-Arg-Pro-D-Ala-NH₂ - (Preparation VIIIB, 40 mg of the TFA salt) in 80% aqueous DMF with bis(salicylaldehydato)nickel(II) (7 mg) in the presence of sodium acetate (4 mg) for 24 hours. The reaction mixture was then subjected to purification by HPLC using solvent system iv in a linear gradient mode (30-50% B). The pure product, [Ac-D-Nal(2)¹,D-Phe(4Cl)²,D-Trp³,Arg⁵,D-Lys [(Ni⁺⁺)(SAL)₂(DL-A₂pr)⁶,D-Ala¹⁰]LHRH (34 mg) has HPLC retention time of 11.1 min when using solvent system iv in a linear gradient mode as above.

50

EXAMPLE XVI

55 pGlu-His-Trp-Ser-Tyr-D-Lys[(Cu⁺⁺)(POLP)₂(DL-A₂bu)]-Leu-Arg-Pro-Gly-NH₂ was prepared by reacting [D-Lys(DL-A₂bu)⁶]LHRH (Preparation VB, 36 mg of the HCl salt) in DMF (0.2 ml) at pH 8 (adjusted with sodium hydroxide and sodium acetate) with pyridoxal-5-phosphate monohydrate (Aldrich, 6 mg) then, after standing at room temperature for 1 hour, with cooper(II) acetate (3 mg in 0.05 ml water). The crude product

was purified by HPLC using solvent system III in a linear gradient mode (20-50% B) to yield [D-Lys⁺(Cu⁺⁺)-
(POLP)₂(DL-A₂bu)⁶]LHRH (18 mg) with a HPLC retention time of 8.8 min.

5

EXAMPLE XVII

Peptide pGlu-His-Trp-Ser-Tyr-D-Lys[(Cu⁺⁺)(FUR)₂(DL-A₂pr)]-Leu-Arg-Pro-Gly-NH₂ was prepared as described in Example IX with the exception that 2,5-dimethyl-4-hydroxy-3(2H)-furanone (Aldrich, 3 mg) was used in place of 5-chloro salicylaldehyde. The product (15 mg) has HPLC retention time of 21.1 min.

EXAMPLE XVIII

15

Peptide pGlu-His-Trp-Ser-Tyr-D-Lys[(Cu⁺⁺) POL]₂(DL-A₂pr)]-Leu-Arg-Pro-Gly-NH₂ was prepared as described in Example IX with the exception that pyridoxal hydrochloride (Aldrich, 4 mg) was used in place of 5-chloro-salicylaldehyde. The DL-A₂pr-containing product obtained (20 mg) has HPLC retention time of 11.2 min.

20

EXAMPLE XIX

25 Peptide pGlu-His-Trp-Ser-Tyr-D-Lys[(Cu⁺⁺)(POLP)₂(A₂pr)]-Leu-Arg-Pro-Gly-NH₂ was prepared as described in Example XVI with the exception that [D-Lys(A₂pr)²]LHRH (Preparation VI, 36 mg) was used as starting material. [D-Lys[(Cu⁺⁺)(POLP)₂(A₂pr)⁶]]LHRH (20 mg), obtained has a HPLC retention time of 6.4 min.

30

EXAMPLE XX

35 Peptide Ac-D-Nal(2)-D-Phe(4Cl)-D-Pal(3)-Ser-Arg-D-Lys[(Cu⁺⁺)(POLP)₂(DL-A₂pr)]-Leu-Arg-Pro-D-Ala-NH₂ was prepared as described in Example XI with the exemption that pyridoxal 5-phosphate (Aldrich, 6 mg) was used in place of salicylaldehyde. The product has HPLC retention time of 5.4 min.

40

EXAMPLE XXI

45 Peptide pGlu-His-Trp-Ser-Tyr-D-Lys[(Cu⁺⁺)(PEN)₂(A₂pr)]-Leu-Arg-Pro-Gly-NH₂ was prepared by reacting [Lys(A₂pr)⁶]LHRH (Preparation VI, 36 mg) in DMF (0.2 ml) at pH 8 (adjusted with sodium hydroxide and sodium acetate) with methyl 2-oxo-cyclopentanecarboxylate (Aldrich, 3 mg) for 24 hour. Thereafter 0.04 ml 1 M NaOH and copper(II) acetate (3 mg in 0.05 ml water) were added. The crude product was purified by HPLC in solvent system III as in Example VI to yield [D-Lys[(Cu⁺⁺)(PEN)₂(A₂pr)⁶]]LHRH (15 mg) having HPLC retention time of 22.2 min when using solvent system III in a linear gradient mode (30-75% B in 30 min).

50

EXAMPLE XXII

55 Peptide Ac-D-Nal(2)-D-Phe(4Cl)-D-Trp-Ser-Arg-D-Lys[(Cu⁺⁺)(POLP)₂(DL-A₂pr)]-Leu-Arg-Pro-D-Ala-NH₂ was prepared as described in Example XVI with the exception that [Ac-D-Nal(2)¹, D-Phe(4Cl)², D-Trp³, Arg⁴, D-Lys(DL-A₂pr)⁶, D-Ala¹⁰]LHRH (Preparation VIII, 40 mg of a TFA salt) was used as starting material in place of [D-Lys(A₂pr)⁶]LHRH. The crude product was purified by HPLC using solvent system IV in a linear gradient mode (30-60% B) to give [Ac-D-Nal(2)¹, D-Phe(4Cl)², D-Trp³, Arg⁴, D-Lys[(Cu⁺⁺)(POLP)₂(DL-A₂pr)⁶]]LHRH (15 mg).

⁵,D-Ala¹⁰]LHRH (35 mg) with HPLC retention time of 5.8 min).

EXAMPLE XXIII

5

The synthesis of peptide Ac-D-Nal(2)-D-Phe(4Cl)-D-Trp-Ser-Arg-D-Lys[(Cu⁺⁺) (POL)₂(DL-A₂pr)]-Leu-Arg-Pro-D-Ala-NH₂ was performed by reacting [Ac-D-Nal(2)¹, D-Phe(4Cl)², D-Trp³, Arg⁵,D-Lys(DL-A₂pr)⁶, D-Ala¹⁰]LHRH (Preparation VIII, 40 mg of a TFA salt) in DMF (0.2 ml) at pH 8 (adjusted with sodium hydroxide and sodium acetate) with pyridoxal hydrochloride (Aldrich, 4 mg) then, after standing at room temperature for 1 hour, with copper(II) acetate (3 mg in 0.05 ml water). The crude product was purified by HPLC under conditions given in Example XI (system iv) to yield [Ac-D-Nal(2)¹,D-Phe(4Cl)²,D-Trp³,Arg⁵,D-Lys[(Cu⁺⁺) (POL)₂(DL-A₂pr)]⁶,D-Ala¹⁰]LHRH (40 mg) with HPLC retention time of 6.3 min.

15

EXAMPLE XXIV

Peptide Ac-D-Nal(2)-D-Phe(4Cl)-D-Pal(3)-Ser-Arg-D-Lys[(Cu⁺⁺)(POL)₂(DL-A₂pr)]-Leu-Arg-Pro-D-Ala-NH₂ was prepared as described in Example XXIII with the exception that [Ac-D-Nal(2)¹,D-Phe(4Cl)², D-Pal(3)³, Arg⁵, D-Lys(DL-A₂pr)⁶, D-Ala¹⁰]LHRH (Preparation VIII, 40 mg of the TFA salt) was used as starting material in place of the corresponding D-Trp³ analog to yield the desired compound, [Ac-D-Nal(2)¹, D-Phe(4Cl)², D-Pal(3)³, Arg⁵, D-Lys[(Cu⁺⁺) (POL)₂(DL-A₂pr)]⁶, D-Ala¹⁰]LHRH (35 mg) with HPLC retention time of 6.4 min.

25

EXAMPLE XXV

Peptide pGlu-His-Trp-Ser-Tyr-D-Lys[(Cu⁺⁺)(POL)₂(DL-A₂bu)]-Leu-Arg-Pro-Gly-NH₂ was prepared by reacting [D-Lys(DL-A₂bu)⁶]LHRH (Preparation VB, 36 mg of the HCl salt) in DMF (0.2 ml) at pH 8 (adjusted with sodium hydroxide and sodium acetate) with pyridoxal hydrochloride (Aldrich, 4 mg) then, after standing at room temperature for 1 hour, with copper(II) acetate (3 mg in 0.05 ml water). The crude product was purified by HPLC under conditions given in Example VI (solvent system iii) to yield the desired product with a HPLC retention time of 11.6 min (system iii).

35

EXAMPLE XXVI

40 Peptide pGlu-His-Trp-Ser-Tyr-D-Lys[(Cu⁺⁺)(SAL)₂(DL-A₂bu-Eac)]-Leu-Arg-Pro-Gly-NH₂ was prepared as described in Example VI with the exception that [D-Lys(A₂pr)⁶]LHRH (Preparation XB, 38 mg of the HCl salt) was used in place of [D-Lys(DL-A₂pr)⁶]LHRH. The desired product has HPLC retention time of 10.4 min. under conditions given in Example VI.

45

EXAMPLE XXVII

Peptide Ac-D-Nal(2)-D-Phe(4Cl)-D-Pal(3)-Ser-Arg-D-Lys[(PtCl₂)-A₂bu-Eac]-Leu-Arg-Pro-D-Ala-NH₂ was prepared as described in Example III with the exception that [Ac-D-Nal(2)¹, D-Phe(4Cl)², D-Pal(3)³, Arg⁵, D-Lys(A₂bu-Eac)⁶,D-Ala¹⁰]LHRH (Preparation XVB, 35 mg) was used as starting material in place of [D-Lys(A₂pr)⁶]LHRH. The desired peptide Pt-complex obtained (10 mg.) has a HPLC retention time of 31.2 min under the conditions of Example III

55

EXAMPLE XXVIII

Peptid Ac-D-Nal(2)-D-Phe(4Cl)-D-Pal(3)-Ser-Arg-D-Lys[(Cu⁺⁺)(SAL)₂(A₂bu-Eac)]-Leu-Arg-Pro-D-Ala-NH₂ was prepared by reacting [Ac-D-Nal(2)¹,D-Phe(4Cl)²,D-Pal(3)³,Arg⁵,D-Lys(A₂bu-Eac)⁶,D-Ala¹⁰]LHRH (Preparation XVB, 35 mg) in 80% aqueous DMF (0.2ml) at pH 8 (adjusted with sodium hydroxide and sodium acetate) with salicylaldehyde (2.7 mg) then, after standing for 1 hour at room temperature, with 5 copper acetate (4.2 mg in 0.05 ml water) for 24 hours. The reaction mixture was then subjected to purification by HPLC with solvents A: 0.1 M ammonium acetate (pH 7) and B: isopropanol (referred to as system iv) using gradient elution (30-80% B in 45 min). Pure Ac-D-Nal(2)¹,D-Phe(4Cl)²,D-Pal(3)³,Arg⁵, D-Lys[(Cu⁺⁺)(SAL)₂(A₂bu-Eac)⁶,D-Ala¹⁰]LHRH (25 mg) has a HPLC retention time of 11.0 min. when using solvent system iv in a linear gradient mode (45-90% B in 30 min).

10

EXAMPLE XXIX

15 The biological activities of and the receptor binding potencies of the claimed compounds are summarized in Table 1 to Table 3.

Table 1 shows the hormonal activity of the compounds of this invention having LHRH agonistic properties as compared to that of LHRH in dispersed rat pituitary cell superfusion system *in vitro* [S. Vigh and A. V. Schally, Peptides 5, 241-247 (1984)]. The peptide was infused for 3 minutes at various 20 concentration, and the amount of LH released was compared to that released by 3 nM LHRH.

Table 2 presents the antiovulatory activity of the claimed compounds having LHRH-inhibiting properties. The inhibitory action was determined *in vivo*, in 4-day cycling rats as described [A. Corbin and C. W. Beattie, Endocr. Res. Commun., 2, 1-23 (1975)]. Affinities of the claimed compounds to membrane receptors of rat pituitary and human breast cancer cells are also given in Tables 1 and 2 as determined by 25 using [¹²⁵I,D-Trp⁶]LHRH.

EXAMPLE XXX

30

To obtain information about the cytotoxic properties of the compounds, the inhibition of ³H-thymidine incorporation into DNA of monolayer cultures of the rat mammary tumor cell line MT-4 was studied. Experiments started with 100 K MT-4 cells/plate on day 0. Dose was changed 3 times, total incubation time with the sample was 5 days. 1μCu ³H-thymidine/ml was added on day 4. Data obtained with the compound 35 of Example VII (Table 3) indicate a strong inhibitory effect even at a concentration of 6x10.⁻¹⁰ M.

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Table 1.

Hormonal activities and receptor binding potencies of agonistic analogues of LHRH containing cytostatic moieties.				
LHRH anal of Ex	Hormonal activity* LH responses rel. to LHRH (= 1)	Binding affinity, for membrane pituitary	K_{d1} , nM ⁻¹ ** receptors of cancer cells	
10	I	133	10.697	6.244
	III	55	3.285	1.743
	VIA	12	3.051	1.348
	VII	10	7.968	3.589
	VIIIA	12	9.710	5.234
	VIIIB	10	6.457	3.334
15	IX	10	8.625	3.995
	X	4	4.513	2.236
	XIV	25	5.589	1.828
	XVI	1.5	9.751	5.038
	XVII	10	1.770	0.536
	XVIII	9	5.817	3.005
20	XIX	0.3	8.876	4.528
	XXI	10.6	10.989	5.096
	XXV	5.5	6.405	3.141

25 *LH responses to the analogues were determined in dispersed rat pituitary cell superfusion system.

30 **Affinity constants of the peptides for the first receptor class (i.e. for high affinity binding sites) of pituitary of male rats and human breast cancer cells, respectively, as determined by using ¹²⁵I-labeled [D-Tr⁶]LHRH.

Table 2.

Hormonal activities and receptor binding potencies of antagonistic analogues of LHRH containing cytostatic moieties.				
LHRH analog of Ex	Hormonal Dose $\mu\text{g/rat}$	activity* % Blockade of ovulation	Binding affinity, for membrane pituitary	K_{d1}, nM^{-1} ** receptors of cancer cells
40	II	1.5	100	7.190
	XII	3	20	9.183
	XIII	1.5	100	1.821
	XV	3	20	8.676
	XX	3	80	5.250
	XXII	10	60	2.901
45	XXIII	10	40	7.622
	XXIV	3	50	5.483
	XXVII			2.152
	XXVIII			0.192

55 *LHRH-Inhibiting potencies were assayed in 4-day cyclic rats.

**Affinity constants of the peptides for the first receptor class (i.e. for high affinity binding sites) of pituitary of male rats and human breast cancer cells, respectively, were determined by using ¹²⁵I-labeled [D-rp⁶]LHRH.

Table 3.

Affinities of the claimed peptides containing D-Met ⁶ to membrane receptors of human prostate cancer and Dunning tumor				
LHRH	Human prostate cancer ^a		Dunning tumor ^a	
anal	K _{a1} nM ⁻¹	K _{a2} nM ⁻¹	K _{a1} nM ⁻¹	K _{a2} nM ⁻¹
I	9.66	---	---	5.73
II	0.7	---	1.68	---
IIA	---	0.97	---	25.14
Control: [D-Trp ⁶]-LHRH	0.04	---	1.87	---

^aAffinity constants of the peptides for the first receptor class, K_{a1} (i.e. for high affinity binding sites) and for the second receptor class, K_{a2} (i.e. for low affinity binding sites) of human prostate cancer and rat Dunning tumor, respectively, as determined by using ¹²⁵I-labeled [D-Trp⁶]LHRH.

Table 4.

Inhibitory effect of compound of Example VII on ³ H-Thymidine incorporation into DNA				
Dose ng/ml	DME Medium + 10% Serum		DME Medium + 0% Serum ^a	
	DPM/Well ^b		DPM/Well ^b	
	x1000	%Inhibition	x1000	%Inhibition
0	102 +/-20.3	--	421 +/-15	--
1 ^c	48 +/-8.6	53	249 +/-30.5	41
10	40 +/-6.7	61	242 +/-32.1	42
100	42 +/-10.4	59	186 +/-13.2	56
1000	38 +/-5.1	65	204 +/-40.9	52
2000	39 +/-4.8	62	249 +/-45.1	41

^aDME medium plus additives, no serum added.

^bMean +/- standard error;

* = .05, ** = .01 by Duncan's new multiple range test.

^ccorresponds to 6x10⁻¹⁰M

Claims

- 50 1. A peptide selected from the group of peptides having the formula:
X-R¹-R²-R³-Ser-R⁵-R⁶(Q)-Leu-Arg-Pro-R¹⁰-NH₂
wherein
R¹ is pGlu, Pro, D-Nal(2), or D-Phe(4Cl),
R² is His or D-Phe(4Cl),
55 R³ is Trp, D-Trp or D-Pal(3),
R⁵ is Tyr or Arg,
R⁶ is D-Phe or R⁶,
where R⁶ is D-Om, D-Lys or D-Phe(NH₂).

- R¹⁰ is Gly or D-Ala,
 X is hydrogen, a lower alkanoyl group of 2-5 carbon atoms or carbamyl,
 Q is bis-(2-chloroethyl)amino group provided that R⁶ is D-Phe,
 where R⁶ is R⁶,
 5 Q is a complexed metal-containing acyl group having the formula:

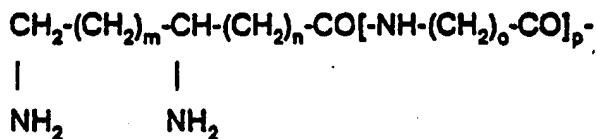
[(Q')(A)] or [(Q')(B)₂(A)]

II III

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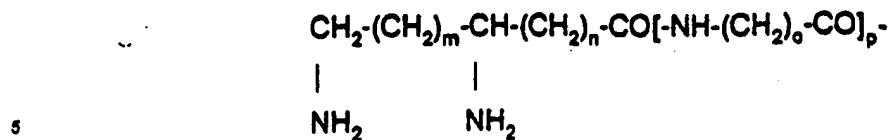
- wherein
 Q' is Pt(Y)₂, where Y is an anion of a pharmaceutically acceptable acid,
 15 A is a diaminoacyl group having the formula

20



where

- 25 m is 0 or 1,
 n and p are 0-10,
 o is 1-10,
 Q' is a non-platinum-group metal, selected from the group consisting of gallium, germanium, tin, titanium, vanadium, iron, copper, cobalt, gold, nickel, cadmium and zinc,
 30 Ba is aralkyldene, heteroaralkyldene, cycloalkyldene or heterocycloalkyldene selected from the group consisting of phenyl loweralkyldenes substituted by halo or nitro; lower alkylpyridyl lower alkylidenes, substituted by hydroxy methyl or phosphoxymethyl; (R₁₂)_q furanyl lower alkylidenes and (R₁₂)_q cycloalkyl lower alkylidines of 5 to 7 carbon atoms in the ring where R₁₂ is lower alkyl and q is 0, 1 or 2; all of the above cyclic moieties being substituted by oxy or carboxy groups, and pharmaceutically acceptable salts thereof
 35 2. A peptide of Claim 1 wherein
 R⁶ is D-Phe and
 Q is bis(2-chloroethyl)amino group.
 3. The peptide of Claim 2 which is
 40 p-Glu-His-Trp-Ser-Tyr-D-Met-Leu-Arg-Pro-Gly-NH₂.
 4. A peptide of Claim 2 which is
 Ac-R¹-D-Phe(4Cl)²-R³-Ser-R⁵-D-Met-Leu-Arg-Pro-R¹⁰-NH₂,
 wherein
 R¹ is D-Nal(2) or Pro,
 45 R³ is D-Trp or D-Pal(3),
 5. A peptide of Claim 1 wherein
 R⁶ is R⁶,
 Q is a complexed metal-containing acyl group having the formula:
 [(Q')(A)]
 50 6. A peptide of Claim 5 which is
 pGlu-His-Trp-Ser-Tyr-R⁶[PtY₂(A)]-Leu-Arg-Pro-Gly-NH₂,
 wherein
 R⁶ is D-Lys,
 Y is Cl,
 55 A is a diaminoacyl group having the formula



where

m is 0 or 1,

10 n is 0,

o is 5,

p is 0-2.

7. A peptide of Claim 5 which is

Ac-R¹-D-Phe(4Cl)-R³-Ser-R⁵-R⁶[(Pty₂)(A)]-Leu-Arg-Pro-R¹⁰-NH₂

15 wherein

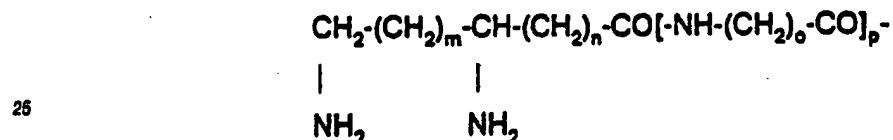
R¹ is D-Nal(2) or Pro,

R³ is D-Trp or D-Pal(3),

Y is Cl,

A is a diaminocarbonyl group having the formula

20



where

30 m is 0 or 1,

n is 0,

o is 5,

p is 0-2.

8. A peptide of Claim 1 wherein

35 R⁶ is R⁶,

Q is a complexed metal-containing acyl group having the formula:

{(Q')ⁿ(B)₂(A)}

9. A peptide of Claim 8 which is

pGlu-His-Trp-Ser-Tyr-R⁶[(Q')ⁿ(B)₂(A)]-Leu-Arg-Pro-Gly-NH₂,

40 wherein

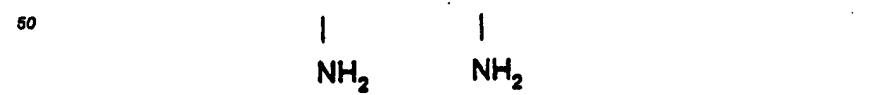
R⁶ is D-Lys,

Q' is copper or nickel,

B is (G)-2-O-^m-benzylidene where G is hydrogen, fluoro, chloro or nitro, (R₁₂)-5,6-dihydro-4-O-^m-3-furylidene where R₁₂ is methyl or hydrogen and q is 0, 1 or 2, (R₁₂)-2-COO-^m-cyclopentylidene where R₁₂ is methyl or

45 hydrogen and q is 0, 1 or 2, or (J)-3-O-^m-4-picolylidene,

A is a diaminocarbonyl group having the formula



55 where

m is 0 or 1,

n is 0,

o is 5,

p is 0-2.

10. A peptide of Claim 8 which is Ac-R¹-D-Phe(4Cl)-R³-Ser-R⁵-R⁶[(Q'')(B)₂(A)]₂-Leu-Arg-Pro-R¹⁰-NH₂, wherein

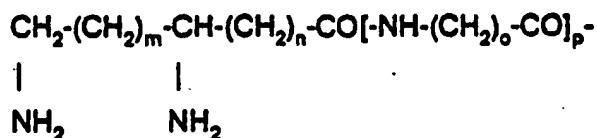
R⁶ is D-Lys,

5 Q' is copper or nickel,

B is (G)-2-O-¹⁸benzylidene where G is hydrogen, fluoro, chloro or nitro, (R₁₂)-5,6-dihydro-4-O-¹⁸3-furylidene where R₁₂ is methyl or hydrogen and q is 0, 1 or 2, (R₁₂)-2-COO-¹⁸cyclopentylidene where R₁₂ is methyl or hydrogen and q is 0, 1 or 2, or (J)-3-O-¹⁸4-picolylidene,

A is a diaminoacyl group having the formula

10



15

where

m is 0 or 1,

20 n is 0,

o is 5,

p is 0-2.

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(54) LHRH analogs.

(55) The present invention deals with LHRH analogues which contain cytotoxic moieties and have influence on the release of gonadotropins from the pituitary gland of mammals, including humans. The compounds of this invention are represented by the formula:

X-R¹-R²-R³-Ser-R⁵-R⁶(Q)-Leu-Arg-Pro-R¹⁰-NH₂

wherein

R¹ is pGlu, Pro, D-Nal(2), or D-Phe(4Cl),

R² is His or D-Phe(4Cl),

R³ is Trp, D-Trp or D-Pal(3),

R⁵ is Tyr or Arg,

R⁶ is D-Phe or R⁶, where R⁶ is D-Orn, D-Lys or D-Phe(NH₂),

R¹⁰ is Gly or D-Ala,

X is hydrogen, a lower alkanoyl group of 2-5 carbon atoms or carbamyl,

Q is bis-(2-chloroethyl)amino group provided that R⁵ is D-Phe,

where R⁵ is R⁶,

Q is a complexed metal-containing acyl group having the formula:



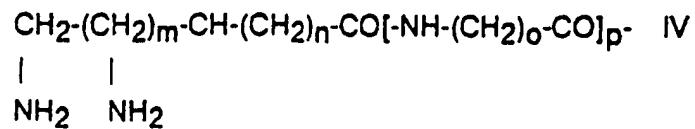
II III

wherein

Q' is Pt(Y)₂, where Y is an anion derived from a pharmaceutically acceptable acid,

A is a diaminoacyl group having the formula

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where

m is 0 or 1,

n and p are 0-10,

o is 1-10,

Q is a non-platinum-group metal, either a main-group metal such as gallium, germanium, and tin, or a transition metal such as titanium, vanadium, iron, copper, cobalt, gold, nickel, cadmium and zinc,

B is a aralkylidene, heteroaralkylidene, cycloalkylidene or heterocycloalkylidene group containing oxygen anion or carboxylate anion at position 2 or 3, and pharmaceutically acceptable salts thereof and methods of use pertaining these compounds.



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REPORT

Application Number

EP 89 11 8460

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
A	INT. J. PEPT. PROT. RES., vol. 32, no. 1, 1988, pages 56-63; A. RICOUART et al.: "Photosubstitution of cymantrenylalanine as a tool in peptide chemistry" * Pages 56-57, column 1 * - - -	1,5-10	C 07 K 7/20 A 61 K 37/43
D,A	CHEMICAL ABSTRACTS, vol. 94, 1981, page 49, abstract no. 11195j, Columbus, Ohio, US; K. CHANNABASAVAIAH et al.: "New potent agonist and antagonist analogs of luteinizing hormone releasing hormone", & PEPT., STRUCT. BIOL. FUNCT., PROC. AM. PEPT. SYMP., 6TH 1979, 803-6 - - -	1-4	
A	J. ENDOCRINOL. INVEST., vol. 11, 1988, pages 535-567; B.J.A. FURR et al.: "Luteinizing hormone-releasing hormone and its analogues: a review of biological properties and clinical uses" * Page 540, column 2 - page 544, column 1 * - - -	1-10	
P,X	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE USA, vol. 86, no. 16, August 1989, pages 6313-6317, Washington, DC, US; S. BAJUSZ et al.: "Highly potent metallopeptide analogues of luteinizing hormone-releasing hormone" * Entire article * - - -	1,5-10	TECHNICAL FIELDS SEARCHED (Int. Cl.5)
P,X	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE USA, vol. 86, no. 16, August 1989, pages 6318-6322, Washington, DC, US; S. BAJUSZ et al.: "Highly potent analogues of luteinizing hormone-releasing hormone containing D-phenylalanine nitrogen mustard in position 6" * Entire article * - - - -	1-4	C 07 K A 61 K

The present search report has been drawn up for all claims

Place of search	Date of completion of search	Examiner
The Hague	28 November 90	GROENENDIJK M.S.M.

CATEGORY OF CITED DOCUMENTS
X: particularly relevant if taken alone
Y: particularly relevant if combined with another document of the same category
A: technological background
O: non-written disclosure
P: intermediate document
T: theory or principle underlying the invention

E: earlier patent document, but published on, or after the filing date
D: document cited in the application
L: document cited for other reasons
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&: member of the same patent family, corresponding document